REPORT OF THE SCIENTIFIC ADVISORY BOARD for the year 1948

Indian Research Fund Association

New Delhi

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REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR 1948

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
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I. COMPOSITION OF THE SCIENTIFIC ADVISORY BOARD.

The Scientific Advisory Board consisted of the following:-

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R.B. Dr. A. C. Banerjea, C.I.E., Director of Medical & Health Services, United Provinces, Lucknow.

Major-General A. C. Chatterji, M.B., D.P.H., Dr.P.H., Director of Health Services, West Bengal, Calcutta.

Dr. R. G. Dhayagude, M.D., Dean, Seth G. S. Medical College, Parel, Bombay.

Dr. B. C. Guha, D.Sc., F.N.I., Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta.

Lieut.-Colonel Jaswant Singh, M.B., Ch.B., D.T.M. & H., D.P.H., Director, Malaria Institute of India, Delhi.

R.B. Dr. M. G. Kini, M.D., M.Ch., F.R.C.S.E., F.A.C.S., F.N.I., F.A.Sc., 69, Marine Drive, Bombay.

Dr. V. R. Khanolkar, B.Sc., M.D.(Lond.), Director of Laboratories, Tata Memorial Hospital, Parel, Bombay.

Lieut.-Colonel C. K. Lakshmanan, L.M. & S., M.R.C.S., D.T.M. & H., D.P.H., Director, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. B. Mukerji, D.Sc., M.D., F.N.I., Director, Central Drug Laboratory, 110, Chittaranjan Avenue, Calcutta.

Lieut.-Colonel B. S. Nat, M.D., Ch.B., F.R.C.S., Director, Health Services, East Punjab, Simla.

Dr. (Miss) S. Pandit, W.M.S., Adviser on Maternity & Child Welfare, Directorate-General of Health Services, New Delhi.

Dr. D. N. Roy, M.D., D.Sc., D.T.M., Director, School of Tropical Medicine, Calcutta.

Major-General Sir Sahib Singh Sokhey, B.Sc., M.A.(Edin.), M.D., Ch.B., D.T.M., & H.(Eng.), F.A.S., F.N.I., Director, Haffkine Institute, Bombay.

Dr. B. B. Yodh, M.B., M.R.C.P., D.T.M. & H., 'Samudra Villa', Darbshah Road, Napien Sea Road, Bombay.

Co-opted member :

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Secretary :

Dr. C. G. Pandit, O.B.E., M.B.B.S., D.P.H., D.T.M., Ph.D., Secretary, Governing Body & Scientific Advisory Board, Indian Research Fund Association, New Delhi.

II. COMPOSITION OF THE ADVISORY COMMITTEES FOR THE YEAR 1949.

I. Cholera.

1. Major-General Sir Sahib Singh Sokhey. (*Chairman*).
2. Lieut.-Colonel M. L. Ahuja.
3. Major-General A. C. Chatterji.
4. Dr. B. B. Dikshit.
5. Dr. J. C. Gupta.
6. Rao Bahadur Dr. R. M. Mathew.
7. Dr. C. G. Pandit.
8. Dr. S. R. Pandit.
9. Dr. D. L. Shrivastava.
10. Dr. K. V. Venkatraman. (*Secretary*).

II. Malaria.

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2. Dr. B. Ananthaswamy Rao.
3. Rai Bahadur Dr. A. C. Banerjea.
4. Bt.-Colonel Sir Ram Nath Chopra.
5. Dr. M. O. T. Iyengar.
6. Dr. K. V. Krishnan.
7. Dr. P. Kumaran Nair.
8. Lieut.-Colonel B. S. Nat.
9. Dr. D. N. Roy.
10. Dr. D. K. Viswanathan.
11. Dr. K. S. Viswanathan.
12. Dr. I. M. Puri. } (*Joint Secretaries*).
13. Dr. S. P. Ramakrishnan. }

III. Nutrition.

1. Dr. G. Sankaran. (*Chairman*).
2. Mr. B. N. Adarkar.
3. Professor S. P. Agharkar.
4. Dr. S. Banerji.
5. Dr. K. P. Basu.
6. Professor M. Damodaran.
7. Dr. B. C. Guha.
8. Dr. (Miss) R. Karnad.
9. Dr. C. O. Karunakaran.
10. Dr. R. B. Lal.
11. Dr. K. Mitra.
12. Professor V. Subrahmanyan.
13. Dr. V. N. Patwardhan. (*Secretary*).

IV. Plague.

1. Major-General Sir Sahib Singh Sokhey. (*Chairman*).
2. Dr. S. C. Ghosal.
3. Dr. A. N. Goyle.
4. Lieut.-Colonel S. D. S. Greval.
5. Dr. V. R. Khanolkar.

6. Dr. R. B. Lal.
7. Dr. C. G. Pandit.
8. Dr. M. Sharif.
9. Dr. K. V. Venkatraman.
10. Dr. P. M. Wagle. (*Secretary*).

V. Clinical Research.

1. Dr. V. R. Khanolkar. (*Chairman*).
2. Dr. R. N. Choudhuri.
3. Dr. R. N. Cooper.
4. Dr. C. R. Das Gupta.
5. A. N. Goyle.
6. Dr. S. W. Hardikar.
7. R.B Dr. M. G. Kini.
8. Dr. P. Kutumbiah.
9. Lieut.-Colonel S. Narain.
10. Dr. M. V. Radhakrishna Rao.
11. Dr. J. C. Ray.
12. Dr. B. B. Yodh.
13. Dr. J. C. Patel. (*Secretary*).

VI. Rabies.

1. Lieut.-Colonel M. L. Ahuja. (*Chairman*).
2. Dr. K. Banerjee.
3. Dr. C. B. d'Silva.
4. Lieut.-Colonel S. D. S. Greval.
5. Dr. S. R. Pandit.
6. Dr. R. Sanjiva Rao.
7. Dr. N. Veeraraghavan.
8. Dr. K. V. Venkatraman.
9. Dr. A. G. Brooks. (*Secretary*).

VII. Leprosy.

1. Dr. C. G. Pandit. (*Chairman*).
2. Lieut.-Colonel A. N. Chopra.
3. Dr. R. G. Cochrane.
4. Dr. P. V. Gharpure.
5. Dr. V. R. Khanolkar.
6. Dr. Dharmendra. (*Secretary*).

VIII. Filariasis.

1. Dr. C. G. Pandit. (*Chairman*).
2. Dr. R. G. Dhayagude.
3. Dr. B. B. Dikshit.
4. Dr. Sangam Lal.
5. Dr. A. K. Mukerji.
6. Dr. C. K. Prasada Rao.
7. Dr. D. N. Roy.
8. Lieut.-Colonel Jaswant Singh.
9. Dr. M. O. T. Iyengar. (*Secretary*).

IX. Industrial Health.

1. Lieut.-Colonel C. K. Lakshmanan. (*Chairman*).
2. Dr. Adisubramaniyam.
3. Cr. B. C. Das Gupta.
4. Mr. S. C. Joshi.
5. Dr. C. L. Katial.
6. R.B. Dr. M. G. Kini.
7. Mr. N. S. Mankiker.
8. Lieut.-Colonel T. C. Puri.
9. Major V. M. Albuquerque.
10. Professor K. Subrahmanyan.
11. Dr. M. N. Rao. (*Secretary*).

X. Maternity and Child Welfare

1. Dr. J. Jhirad. (*Chairman*).
2. Dr. A. Benjamin.
3. Dr. G. Coelho.
4. Miss M. Craig.
5. Dr. C. R. Das Gupta.
6. Dr. N. A. Purandare.
7. Dr. D. M. Satur.
8. Dr. Muktha Sen.
9. Dr. S. Pandit. (*Secretary*).

XI. Pharmacology.

1. Bt.-Colonel Sir Ram Nath Chopra. (*Chairman*).
2. Dr. J. C. Gupta.
3. Dr. B. B. Dikshit.
4. Dr. B. N. Ghosh.
5. Dr. B. Mukerji.
6. Dr. B. B. Yodh.
7. Mr. P. M. Nabar. (*Secretary*).

III. MEETINGS HELD BY THE SCIENTIFIC ADVISORY BOARD IN 1948.

The Board held its annual meeting on the 3rd and 4th December, 1948, and confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers. It also considered in detail the programme of research for the year 1949-50 and recommended the programme given on pages 240 to 245 of this Report.

Technical Report of the Scientific Advisory Board of the Indian Research Fund Association for the year 1948.

IV. RESEARCHES CARRIED OUT DURING THE YEAR.

The researches which were carried out during the year under report were recommended by the Board at its meeting held in New Delhi on the 12th and 13th December, 1947 and were approved by the Governing Body of the Indian Research Fund Association at its meeting held in New Delhi on the 20th March, 1948.

CHOLERA.

1. Enquiry on cholera under the Director, School of Tropical Medicine, Calcutta.

I. *Biochemistry of cholera cases.*—Chloride content of the whole blood of clinical cases of cholera was estimated on admission to the hospital before the injection of saline and during the convalescence. The results are shown in the following table. Stools of all the cases were examined by culture.

	No. of cases.	Blood chloride in mg. per 100 c.c.	
		Before saline.	During convalescence.
Bacteriologically-proven positive cases ...	31	425±16·6	450±6·7
Bacteriologically-proven negative cases	135	425± 6·8	448±5·4

The results were statistically analysed. No significant difference in the chloride content of the blood either in the bacteriologically-proven positive or negative cases before injections of saline and during the convalescence could be observed.

II. *Preparation of monospecific sera.*—Preparation of monospecific agglutinating sera for Inaba, Ogawa and Inaba rough types of *V. choleræ* was undertaken by this enquiry. Approximately 50 c.c. each kind of sera of satisfactory titre have been available for distribution and supplied to several firms in Calcutta. In pursuance of the report of the World Health Organization recommending the establishment of preference preparation of Inaba and Ogawa monospecific agglutinating sera to be prepared in Inaba and held for distribution at the State Serum Institute at Copenhagen, it has been reported that serum can be supplied on request.

III. *Trial of drugs.*—(a) *Formocibazol:* This was received on the 30th June 1948 and the trial commenced on the 3rd July, 1948. This was the declining period of the epidemic. The drug was administered to alternate cases on admission into hospital. All of them were advanced cases and saline injection had, therefore, to be given to all of them. The drug was administered in the following doses: Six g. on admission and 4 g. 4 hours later. Two doses of 4 g. on the second day. Thereafter, 2 doses of 1 g. morning and evening for the next five days. The results of the trial are given below:—

	Number of cases.	Deaths.
Treated with Formocibazol ...	40	3
Control ...	40	7

Stools of all the cases were examined by culture and their results are classified as follows :—

	Bacteriologically proven:			
	Positive cases.		Negative cases.	
	Number of cases.	Deaths.	Number of cases.	Deaths.
Treated with Formocibazol ...	8	3	32	0
Control	10	3	30	4

The results are not conclusive.

(b) *Uppu Chunnam.*—Uppu Chunnam was given in clinical cases of cholera. Selection of cases was on alternate basis of admission. All of these cases were advanced cases and saline had to be given to all of them. The experimental group was divided into two subgroups according to the doses administered. The drug was administered in warm water. Eight doses were given in all cases. The results are shown below :—

TABLE I.

	Cases.	Deaths.
Uppu Chunnam (4 gr. 4-hourly)	8	2
Control	8	1

TABLE II.

Uppu Chunnam (2 gr. every 15 min.)	8	2
Control	8	0

TABLE III

	No. of cases passing vibrios.	
Uppu Chunnam (4 gr. 4-hourly)	6	1
Control	2	0

TABLE IV.

Uppu Chunnam (2 gr. every 15 min.)	6	2
Control	4	0

The results were not encouraging.

2. Cholera treatment unit under the Director, King Institute, Guindy, Madras.

The trials of sulphaguanidine, sulfanilamide formalin precipitate and sulphathalidine in the treatment of hospitalized cases of cholera receiving adequate saline transfusion and other supportive treatment referred to last year were concluded. The trial of these drugs was made consecutively and not concurrently and, therefore, represent their administration in different phases of the epidemic. The variation in the mortality in control cases

undergoing routine treatment which was the same throughout the entire period represents the natural variation in the mortality during the epidemic. The results of the administration of these drugs is shown in the Table :—

TABLE

Drug.	Period of trial	Number of cases* treated	Mortality	Number of control cases.	Mortality.
Sulphaguanidine	1–10–1947 to 16–11–1947	138	21(15·22%)	136	18(13·2%)
Sulfanilamide formalin precipitate	16-11-1947 to 18–12–1947	113	49(42·6%)	95	24(25·3%)
Sulphathalidine	18-12-1947 to 25–1–1948	286	67(23·4%)	328	77(23·5%)

*Only cases in which the diagnosis was confirmed by the isolation of *V. cholerœ* are included.

A detailed analysis of the data obtained will be presented in a paper to be published.

The routine serological examination of strains of *V. cholerœ* isolated at the Institute was continued. Of 1,829 *V. cholerœ* strains examined, 1,792 were Inaba and 37 Ogawa.

In response to a request from Dr. Bruce White of the Medical Research Council, London for specialized material for study, about 60 cases of cholera were followed through their entire course and vibrios isolated at different stages and filtrates of stools were secured and transmitted to him.

A number of strains of recently isolated vibrios were screened for their mouse pathogenicity as a preliminary to the selection of strains of adequate antigenicity for incorporation in routine prophylactic vaccine manufacture.

3. Enquiry on cholera under the Director, Central Research Institute, Kasauli.

The object of the present cholera enquiry at the Central Research Institute, Kasauli, is the isolation and study of the active immunizing fraction or fractions from *V. cholerœ*. For this purpose polysaccharide fractions were prepared from various strains in the following ways :—

1. Bruce White's method from growth on agar.
2. Phenol method from growth on agar.
3. Alcoholic precipitation method from the growth in a liquid medium.

In all the three methods the chief precipitant of the polysaccharide after it has been set free as a result of the breakdown of the bacterial bodies, is ethyl alcohol. They differ in their ways of getting rid of protein, which is done in method (1) by treatment with saturated picric acid, in method (2) by treatment of dried bacteria with 90 per cent phenol and in the last method by shaking with chloroform and butyl alcohol or amyl alcohol in the presence of sodium acetate and acetic acid. Inorganic salts, mainly phosphates are removed by repeated precipitations with alcohol in acidic solution, and also if necessary,

by precipitating the chilled solution with cold glacial acetic acid. The preparations are finally isolated by precipitating with absolute alcohol with the addition of a minimum amount of 40 per cent sodium-acetate solution. The precipitate is washed thrice with absolute alcohol, filtered under suction and finally dried in vacuo at about 50 C. The dried product is soluble in distilled water or 0·85 per cent sodium-chloride solution. With certain preparations molisch was positive up to a dilution of 1 in 100,000. All the preparations were biuret and phosphate negative. They reacted with the precipitin sera prepared against Inaba and Ogawa subtypes, in certain cases up to a dilution of 1 in 1,000,000 and none of the preparations showed any definite subtype specificity.

Sixteen representative fractions were tested for their immunizing efficiency in mice. Two doses of the fractions were given subcutaneously into mice of 15 g. to 20 g. weight at intervals of a week followed a week later by injection of 10, 100 and 1,000 challenge doses of mucinized suspensions of *V. cholerœ*. All the three methods of preparation gave fractions that offered varying degree of protection to mice against test infection but the best fraction was obtained by the phenol method. The immunizing efficiency of this fraction has been studied in greater detail and the minimum dose of the antigen required to protect 50 per cent of the mice immunized with it was determined.

The results obtained so far are highly encouraging and it is proposed to continue the work during the next year.

REPORT OF THE MEETING OF THE CHOLERA ADVISORY COMMITTEE.

Held in the Senate House, Madras, on the 30th November, 1948 at 2-30 p.m.

PRESENT.

Major-General Sir Sahib Singh Sokhey. (*Chairman*).
Lieut.-Colonel M. L. Ahuja.
Major-General A. C. Chatterji.
Dr. B. B. Dikshit.
Dr. J. C. Gupta.
Rao Bahadur Dr. R. M. Mathew.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Dr. K. V. Venkatraman. (*Secretary*).

Dr. D. L. Shrivastava was unable to attend.

Dr. K. C. K. E. Raja, Chairman, Scientific Advisory Board, was present.

The following were also present :—

Dr. A. R. Mehta.
Dr. Lieut.-Col. Barkat Narain.
Dr. Lieut.-Col. B. N. Hajra.
Dr. Gurkirpal Singh.
Dr. K. P. Thomas.
Dr. N. K. Tampi.
Dr. P. M. Wagle.
Dr. R. Ananthaswami Pillai.
Dr. S. S. Pillai.

Major-General Sir Sahib Singh Sokhey in opening the proceedings of the Committee made a reference to the report of the Expert Committee on Biological Standardization included in item 3 of the agenda and Dr. Pandit to his note on the work done by the Study Group of Cholera constituted jointly by the Office International d'Hygiene Publique and the World Health Organization. It was indicated that a more detailed consideration of these items and the suggestions made would follow at a later stage, and the suggestion was made that the recommendation on the continuance of the existing enquiries and the proposed new enquiry may be deferred till then.

2. The Committee considered the work carried out by the following enquiries :—

(i) *Enquiry on cholera under the Director, School of Tropical Medicine, Calcutta.*

Dr. J. C. Gupta presented the report of the work done by the unit previously circulated.

(ii) *Cholera treatment unit under the Director, King Institute, Guindy, Madras.*

Dr. K. V. Venkatraman presented the report on the work done by the unit. He also referred to the results obtained in a trial of Formol Cibizol at the Infectious Diseases Hospital, Tondiarpet, Madras, subsequent to the submission of the note. Of 138 cases in whom the diagnosis was established by the isola-

tion of *V. cholera*, 75 cases were treated with Formol Cibizol and 63 were under the routine hospital treatment. The case mortality in the two groups were 7 and 6 respectively, showing no significant difference.

In a very lively discussion which followed, reference was made to a resolution of the Committee in a previous year recommending trials of drugs elaborated by workers of the Association and Dr. B. B. Dikshit wanted to know the circumstances in which the new compound Formol Cibizol was taken up. Dr. Venkatraman stated that following the wide publicity given to this new drug in the lay press, and the very favourable results reported to have been achieved with it in the treatment of a very small and inadequately controlled series of cases, he addressed the Secretary, Indian Research Fund Association, on the desirability of securing a quantity of the drug for an adequately controlled trial by the treatment units of the Association. The Secretary agreed with the suggestion and the representative in India of Ciba Limited placed a generous amount of it at his disposal. The results obtained with this drug in Madras would seem to indicate that in cases receiving saline transfusion and other supportive treatment, the administration of the drug does not confer any more benefit than the other sulpha drugs previously tried and given up.

(iii) *Enquiry on cholera under the Director, Central Research Institute, Kasauli.*

Lieut. Colonel M. L. Ahuja presenting the report of the cholera enquiry at the Central Research Institute, Kasauli, stated that polysaccharide fraction had been prepared from several strains of *V. cholera* by different methods and 16 representative fractions had been tested for their immunizing efficiency in mice. The results with fractions obtained by the phenol method have been encouraging and it is proposed to study the fractions prepared from laboratory altered strain.

3. Major-General Sir Sahib Singh Sokhey referred to the extract from the report of the Expert Committee of the World Health Organization on Biological Standardization and drew the special attention of the Committee to the resolution expressing the hope that all facilities will be given by the health authorities of India to have carefully controlled tests done in the field of the protective action of cholera vaccine of high immunizing potency in animals and containing all the necessary smooth antigens. He also stated that arrangements have been made for the establishment of two reference preparation of cholera vaccine of the Ogawa and Inaba strains. To facilitate the use of the reference preparation for comparative tests of potency, Sir Sahib Singh agreed to prepare freeze-dried living cultures of virulent Ogawa and Inaba strains which would be held by the Central Research Institute, Kasauli, for distribution.

4. Dr. C. G. Pandit then presented his note on the work done by the Study Group in the April and October meetings in Paris (*Annexure*). He drew special attention to the necessity for further study in India and other endemic areas on the factors governing endemicity and the suggestion that teams of epidemiologists and bacteriologists familiar with conditions in one endemic area should be given an opportunity to study an endemic area in other country. He asked that the Committee should consider the desirability of setting up a small committee to prepare a standard procedure for diagnosis of cholera and referred to the re-affirmation of the value of the buffer saline transfusion medium evolved by Dr. Venkatraman working under the Indian Research Fund Association. Dr. Pandit said that the Study Group would hold

its next session in India in 1949, and urged the Committee to formulate proposals for re-orientation of the work during the next year so as to make available information for the solution of many specific problems mentioned in the note.

Dr. K. C. K. E. Raja attached the greatest significance to the change in the approach to the problem of the control of cholera through intensification of effort for the eradication of cholera in endemic areas rather than the quarantine basis alone. He referred to the earlier statistical studies on the epidemiology of cholera in India and expressed the view that India was best suited for the WHO team work on account of extensive previous studies made here and the availability of a number of workers who have been continuously engaged on these investigations. He stated that in view of the importance attached to correct diagnosis, the responsibility for the preparation of monospecific sera and reference preparation of vibrio strains should rest with the Central Research Institute. He stressed the necessity for a re-orientation of the programme of studies and suggested that this could best be done through a sub-committee which could go into all the points raised in Dr. Pandit's note with great care and called on Dr. Pandit to make a proposal.

Dr. Pandit moved and Dr. Raja seconded that a sub-committee (the composition to be determined by the Chairman, Dr. Raja and Dr. Pandit) be formed to make proposals for a re-orientation of the programme of studies on cholera to be undertaken during the next year. This was approved and the continuance of the existing enquiries except the enquiry at the Central Research Institute, Kasauli, in their present form was not recommended. In view of the fundamental nature of work, it was resolved to recommend that the enquiry at the Central Research Institute, Kasauli, be continued and a grant of Rs. 17,533/- be sanctioned for the purpose.

The Secretary, I.R.F.A., was requested to frame the budget in accordance with the proposals to be made by the sub-committee.

ANNEXURE.

Note by Dr. C. G. Pandit on the work done by the Joint OIHP-WHO Study Group on Cholera.

Early in the year the Office International d'Hygiene Publique, Paris, and the World Health Organization jointly constituted several Study Groups to take stock of the existing knowledge in regard to some of the major epidemic diseases which, in view of their likely spread from one country to another, were of considerable international importance. For the present four separate Study Groups were constituted to study the problems in connection with :—

(1) Plague and rickettsial diseases.

(2) Cholera.

(3) Smallpox.

Subsequently in October this year another Study Group was set up for the study of problems connected with Trachoma.

Major-General Sir Sahib Singh Sokhey was a member of the Study Group on Plague and Rickettsial diseases and Dr. C. G. Pandit was a member of the other three groups. This note refers to the work done by the Study Group in connection with Cholera.

The reports of the meetings of the Study Group on Cholera held in June and October, 1948, are submitted for the information of the members of the Cholera Advisory Committee. It will be seen that the Study Group have stressed the necessity of undertaking investigations in areas where cholera is prevalent of several problems where precise knowledge is at present limited. In particular attention is invited to the report of the meeting of the Study Group held on the 21st October, 1948, especially items 4.1, 4.6, 4.7 and 5 of the report. It is for the consideration of the Cholera Advisory Committee whether they should now set up a small committee to prepare a standard procedure for the diagnosis of cholera for submission to the World Health Organization in due course.

The Egyptian representative had submitted for consideration the standard procedure followed in Egypt for the diagnosis of cholera. This, as well as the details of the hæmolytic tests recommended by the Egyptian workers would be made available to the members of the Cholera Advisory Committee in due course.

Attention is also invited to items 7.1, 7.2, and 7.3 of the report of the October meeting of the Study Group. A view was expressed at the meeting that the Study Group should meet next in India, where they would be in a position to consult several experts in the country. Should this decision be accepted, Dr. Pandit informed the Study Group that it would be desirable to hold their meeting at a time when the Cholera Advisory Committee meets annually in India in December. This recommendation would be in consonance with the resolution recorded in item No. 7.2 of the report.

The Cholera Advisory Committee is further requested to consider item 7.3 of this report in order to decide whether any action should be taken immediately in India in the matter.

It is also advisable that the Cholera Advisory Committee should consider the several problems referred to in the two reports and expressed their agreement or otherwise on the recommendations made therein.

EXPERT COMMITTEE ON INTERNATIONAL EPIDEMIC CONTROL.

Summary Report of the OIHP-WHO Joint Study Group on Cholera on its 1st Session

The Study Group met at the Office International d'Hygiene Publique, Paris, from 5th to 7th April, 1948. It included* :—

Dr. C. G. Pandit, Director of the King Institute of Preventive Medicine, Madras.

Dr. Aly Tewfik Shousha Pasha, Secretary of State for Public Health, Cairo.

Dr. P. Bruce White, National Institute for Medical Research, Hampstead, London.

Secretariat :

Dr. Maurice Gaud, Acting Director of the Office International d'Hygiene Publique, led the debates.

Dr. Yves Biraud, Director of Epidemiology and Public Health Statistics, World Health Organization,

assisted by

Dr. W. M. Bonne, Medical Officer, World Health Organization.

With a view to finding what changes were needed in the provisions of International Sanitary Conventions, in the light of recently acquired scientific knowledge, the Study Group considered the following points on its agenda :—

1. Recent distribution of cholera throughout the world.
2. Facts regarding cholera vibrio.
3. Modes of transmission : (a) direct, and (b) indirect (through water, foodstuffs, flies, clothing, etc.).
4. Duration of incubation period.
5. Prophylaxis : (a) General measures.
   (b) Vaccinations (methods employed and results obtained).
   (c) Immunization by bacteriophage.
6. Conservation of the virus during the inter-epidemic periods ; germ carriers : their part in the transmission of the disease and international sanitary measures concerning them.

The Study Group made the following observations and recommendations for further research :—

1. *World distribution of cholera.*—Although cholera is capable of invading the whole world it prevails in a relatively small number of countries of Asia and the areas where it persists constantly are few and limited.

This justifies a study of the truly endemic areas of cholera and the reasons for their remaining so, with a view to finding means for eradication of the disease in those areas, with the technical help of the World Health Organization, if needed.

* Dr. Georges Blanc, Director of the Pasteur Institute of Morocco, Casablanca, and Dr. Atilio Macchiavello, U.S. Public Health Service, Representative of the Pan-American Sanitary Bureau, Lima, Peru, also attended some meetings.

Such an attack on endemic foci would be more effective for the international epidemic control than mere protective measures, taken by individual countries at their borders under International Sanitary Conventions.

2. *Facts concerning the cholera vibrio.*—2.1. The Ogawa and Inaba types of cholera vibrio may be responsible for successive epidemics of equal severity in a particular area and do not have a fixed geographical distribution.

These types do not represent true sub-species as under laboratory conditions the Ogawa type may be transmuted to the Inaba type, though the reverse change has not, up to the present, been observed.

2.2. At the end of the disease and during convalescence an increasing proportion of the vibrios excreted by the patient are in the process of "roughening" or are entirely rough. Transformation from the smooth to the rough state corresponds to a loss of pathogenicity of the organism.

No reversion from the fully rough to the smooth form has so far been observed. Whether vibrios partially rough can recover full smoothness and virulence is not known.

2.2.1. *It is recommended* that a comparison be made of vibrios isolated from persons convalescent from cholera and from persons who have been in contact with cases of cholera, with special reference to their smooth and rough characters, serological type, bacteri-phage resistance or susceptibility and virulence, as determined by the mouse test.

2.2.2. *It is recommended* that an investigation be made of the possibility that some differences in the degree of smoothness or roughness of the vibrio isolated from contacts, have a prognostic bearing on the outcome of the initial infection, i.e. on the development or non-development of cholera following on ingestion of the vibrios.

2.3. In view of the capital importance for both national and international measures to be adopted of early diagnosis of cholera, particularly in areas where its presence is unusual, *investigation is recommended* of: Laboratory procedures relating to isolation and recognition of the cholera vibrio, and particularly to the differentiation of El Tor vibrio from the true cholera vibrios of O group I.

2.3.1. The relative merits or significance.

2.3.1.1. Bandi's test for quick diagnosis of cholera.

2.3.1.2. Copra antigens and antibodies.

2.3.1.3. The haemolytic test with special reference to Greig's technique and the Egyptian modification of the same.

2.3.2.1. The possibility of applying 'phage technique in the diagnosis of cholera vibrio.

2.3.2.2. 'Phage typing of cholera vibrios for epidemiological purposes with special reference to *A* type 'phage. (*cf.* 'phage typing of strains of *Bact. typhi*).

2.3.3. Procedures to be recommended for :—

2.3.3.1. Collection and despatch of material to the laboratory from the field (including ships at sea).

2.3.3.2. Standard laboratory practice for the identification of the cholera vibrio.

3. *Modes of transmission.*—3.1. It should be specified that the export of the following articles will be strictly forbidden :—

3.1.1. Personal linen, rags and clothing recently worn by patients or suspects.

3.1.2. Fresh fish, crustaceans and shell-fish as well as green vegetables eaten raw, coming from contaminated regions.

3.1.3. The export of fruit does not appear to involve any danger if it is in transit for more than five days.

3.2. Ships may not take in drinking water at an infected port.

In cases where this cannot be avoided the water must be disinfected under the supervision of the port sanitary authorities who must take full responsibility for the operation.

4. *Incubation period.*—The period of incubation now fixed at 5 days for purposes of international quarantine in the Sanitary Conventions is too short and should be extended to 6 or 7 days, in accordance with the available data. These data *require* to be supplemented by *further study.*

5. *Prophylaxis of cholera.*—5.1.1. For purposes of International Quarantine Legislation, only infections by the typical *Vibrio choleræ* Koch are to be considered.

5.1.2. " Medical examination " as mentioned in the International Sanitary Conventions must be understood to include such laboratory examinations (including rectal swabbing) as are deemed necessary by the health authorities.

This should be stated explicitly in the definitions to be given in International Sanitary Regulations.

5.1.3. *A study is recommended* of the use of sulphonamides in the prophylaxis of cholera.

5.2. *Vaccination.*—5.2.1.1. Cholera vaccine is of definite value in the prophylaxis of cholera if prepared with strains of real antigenic potency.

5.2.1.2. The immunity it confers is, however, relative and not absolute.

5.2.1.3. Immunity is believed to reach a significant level as early as the 4th day after injection of the vaccine and to attain its full effectiveness by the 8th day.

5.2.1.4. There is reason to believe that it lasts at least six months.

5.2.2.1. One single injection of 1 c.c. of vaccine confers an appreciable immunity and constitutes a proper procedure for mass vaccination campaigns, when further immunization is impracticable.

5.2.2.2. Two injections at a week's interval are, however, preferable and should be given to individuals particularly exposed to infection or having special public responsibility (sanitary, police, military and administrative personnel).

5.2.3.1. There is no contra-indication to cholera vaccination as it gives little or no reaction ; it can be applied at all ages provided care is taken to reduce the dose in proportion to body-weight in the case of infants.

5.2.3.2. *A study is recommended* of the antibody response in children of varying ages from infancy, to graduated doses of cholera vaccine.

5.2.4. Biological standardization of the protective antigenicity of cholera vaccine is when possible and so far as it is possible desirable to ensure its potency as the numeration of germs in the vaccine does not provide a reliable standard of efficacy.

5.2.5. In view of the relative character of the immunity conferred by vaccination, vaccinated persons should not be exempted from all measures of control by the International Quarantine Regulations. Sanitary "surveillance" now provided in the International Sanitary Conventions is, however, considered as an adequate protective measure.

5.2.6. *It is recommended* that a study as to specific agglutinins for Inaba and Ogawa types respectively be made of the antibody content of the serum of :—

5.2.6.1. Convalescents.
5.2.6.2. Contacts.
5.2.6.3. Persons vaccinated.

The determination of the agglutinin content should be correlated to the vibrio type responsible for the epidemic or included in the vaccine administered.

5.3. After reviewing existing knowledge regarding cholera bacteriophage the Study Group was of the opinion that *further investigation* was still desirable of its application in the prophylaxis and treatment of cholera.

6. *Germ carriers.*—6.1. The importance of germ carriers in the transmission of cholera varies considerably according to the type of carriers :—

Sub-clinical cases.
Convalescents.
Healthy carriers.

6.2. Sub-clinical cases and cases at the end of the incubation period constitute the most important agents in the transmission of cholera.

6.3. Convalescents excrete vibrios for a period of about 15 days counted from the onset of the disease. This period may in exceptional cases be a month and even more.

6.4. However, all observers agree that convalescents do *not* play a significant part in the transmission of the disease.

6.5. Healthy germ carriers are usually to be found amongst contacts of confirmed cases : their number decreases with the epidemic and they rapidly disappear after its end. They play only a minor part in the transmission of the disease.

6.6. Nevertheless, for purposes of International Sanitary Regulations a demonstrated carrier, whether a convalescent carrier or not, should be submitted to the measures provided for cholera cases.

6.7. A case or carrier should be freed from all measures after three successive negative bacteriological examinations of his stools or rectal swabs, carried out at intervals of *at least* 24 hours have been made.

6.8. When no stools are available for examination, rectal swabbing is an adequate substitute. The administration of a laxative to obtain stools for examination entails unnecessary discomfort or even risks for the person examined and should not be resorted to.

6.9. *It is recommended* that sulphonamides be tried with a view to determining their efficacy or inefficacy in the sterilization of germ carriers, both convalescent and healthy.

Summary Report on the Second Session of the Joint OIHP-WHO Study Group on Cholera.

The Study Group met at the Office International d'Hygiene Publique, Paris, from 13th to 15th October, 1948. The following were present :—

H.E. Sir Aly T. Shousha Pasha, Under-Secretary of State for Public Health, Cairo ; President of the Executive Board, WHO.

Dr. C. G. Pandit, Secretary, Indian Research Fund Association, New Delhi.
Dr. Bruce White, National Institute for Medical Research, Hampstead, London.
Prof. A. Dujarric De La Riviere, Assistant Director, Pasteur Institute, Paris.
Dr. Atilio Macchiavello, U.S. Public Health Service, Consultant Epidemiologist of the Pan-American Sanitary Bureau, Lima.

Dr. O. Ouchterlony, State Bacteriological Laboratory, Stockolm.
Médecin-Général Inspector Vaucel, Director of the Health Service, Ministry of Overseas Territories, Paris.

The following also attended the meetings :—

OIHP :

Dr. M. Gaud, Director of the Office International d'Hygiene Publique, acted as Chairman.

WHO :

Dr. Y. M. Biraud, Director of the Division of Epidemiology, assisted by Dr. G. Stuart, Chief of the Sanitary Conventions and Quarantine Section.

The Study Group considered the following items on its agenda :—

1. Determination of cholera endemic zones throughout the World.

1.1. *Geographical distribution.*—The Study Group considered cholera prevalence in India, Thailand, Indo-China and China, the only countries in which the disease had recently been reported.

Note was taken that while in certain areas, e.g. in Southern Indo-China, there seemed to be a continuous presence of the disease but with a small number of cases ("hypo-endemicity"), cholera in much higher incidence prevailed uninterruptedly in Bengal. But even there cholera was not constantly present in every district.

1.2. *Fatality rates.*—The Study Group noted the difference in fatality rates between true endemic areas, where they often were as low as 7 to 10 per cent, and non-endemic areas, where during epidemics it reached 50 per cent and over.

1.3. *Definition of endemic areas.*—The Study Group agreed that an endemic area is one in which, over a number of years, there is practically continuous presence of clinical cholera with annual seasonal exacerbations of incidence.

1.4. *Criteria of endemicity.*—In India the following criteria have been suggested for the determination of endemic, non-endemic and intermediate areas :—

1.4.1. *Percentage of months without cholera :—*

Endemic	...	...	Less than 30 per cent.
Intermediate	...	...	30 to 50 per cent.
Non-endemic	...	...	Over 50 per cent.

1.4.2. *Mean length in months of intervals between prevalence of cholera* :—

Endemic	Less than 2·5.
Intermediate	2·5 to 4.
Non-endemic	Over 4.

1.5. *Rates of incidence.*—On the basis of the above criteria, 80 districts in Bengal out of 180 were found to be truly "endemic", none in the Madras Province.

1.6. It was recommended that the WHO Secretariat establish world maps of recent cholera incidence and of cholera endemic areas, the essential criterion for the determination of endemicity being continuity of infection rather than number of persons affected.

2. Factors governing endemicity.

2.1. The Study Group considered that the factors governing endemicity should be investigated on the spot.

2.2. The Study Group took note of researches carried out up to 1942 in India on the vibrios contained in "tanks" and in similar collections of water. There *V. choleræ* Koch, was found only when clinical cases existed in the population using the tanks as their water supplies, but *not* during cholera-free periods.

2.3. The presence of salt and organic matter was essential to the survival of cholera vibrios; without salt, vibrios died within 24 hours. These findings suggested that survival of cholera vibrio in water was not a proved factor in the maintenance of cholera endemicity.

2.4. The Study Group considered that further investigations should be carried out to determine whether the excretion of cholera vibrios from mild sub-clinical cases of cholera could constitute a link between two cholera outbreaks.

2.5. Several methods were suggested which might throw light on the possibility of man, acting during periods apparently cholera-free, as reservoir of the infection.

2.5.1. It was suggested, on the basis of recent Egyptian experience, that in an endemic area a representative group of population be subjected to periodical rectal swabbing for at least one year, in order to detect carriers and establish a possible relation between the carrier state and mild diarrhœal conditions.

2.5.2. It was also suggested that in selected areas, endemic and non-endemic alike, all persons dying without medical attendance and irrespective of the cause of death should, in order that the presence of *V. choleræ* might be detected, be subjected to systematic rectal swabbing.

2.5.3. It was further suggested that teams of epidemiologists and bacteriologists, familiar with conditions in one endemic area such as Bengal, should be given an opportunity to study an endemic area in another country, such as Southern China or Indo-China, and compare conditions in the two areas for the purpose of determining factors governing endemicity.

3. "S" and "R" forms of *V. choleræ*.

3.1. The Study Group considered the results of observations which confirmed the transformation of cholera vibrios from the "Smooth" to the

"Rough" forms during the course of the disease. The appearance of the "Rough" form as from the fourth day apparently corresponded to the development of anti-bodies in the patient's serum.

3.2. In view of the epidemiological importance that a reversion from the "R" into the "S" form would have, if it occurred in nature, the Study Group recommended that this question be further investigated.

4. Diagnosis of cholera.

4.1. The Study Group considered the desirability, for international purposes, of attaining a standard procedure for the diagnosis of cholera.

4.2. The Study Group agreed on the desirability of providing laboratories with standard preparations of the O Inaba and Ogawa antigens with a view to enabling them to prepare qualitatively identical agglutinating sera for diagnostic purposes, thus endorsing the decision taken by the WHO Expert Committee on Biological Standardization.

4.3. It further agreed that it was desirable to furnish with such antigens a general description of the method for their proper employment.

4.4. With a view to providing a standard method of diagnosis, the Group recommended that a comparative study be made in India between the procedures used in Egyptian, Indian and other laboratories.

4.5. The Study Group agreed that, for purposes of International Quarantine, the presumptive diagnostic tests (Bandi's, Copra antigens, etc.) could not suffice to diagnose cholera, in areas where it is not usually present and could not dispense with the use of a classical diagnostic method which might require 48 hours.

The Group recommended a comparative study of the results obtained with Bandi's test and with the classical methods respectively.

4.6. The Study Group recommended a comparative study of the Egyptian technique of the hæmolytic test and of Greigh's technique, using respectively the blood of goats and sheep.

4.7. *Collection and despatch of diagnostic material to the laboratory.*—The Study Group re-affirmed the value of buffer saline solution* in screw-capped glass containers for despatching suspected stools to distant laboratories for diagnosis.

Vibrios have been recovered from stools in such buffer solution over long periods.

5. Incubation period.

After reconsidering the incubation period of cholera, the Study Group very strongly urged that further observations be made on this point in India, before a change could be suggested in the 5 days duration now embodied in the International Sanitary Conventions.

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* *Preparation of solution*: 12·405 g. of boric acid and 14·912 g. of potassium chloride are dissolved in 800 c.c. of hot distilled water. Solution is then cooled and made up to 1 litre. From this stock 250 c.c. are taken and mixed with 155·5 c.c. of N/5 NaOH; the whole is now made up to 1 litre. Twenty g. of sea salt are dissolved and the buffer saline is filtered through paper. Peptone to a concentration of 1 : 50,000 is then added. The buffered saline is dispensed in 10 c.c. quantities in 30 g. (1 oz.) screw-capped bottles and autoclaved. The pH of the medium after autoclaving is about 9·2.

6. Prophylaxis.

6.1. *Vaccination.*—6.1.1. The Study Group was informed of the preliminary results obtained with Sokhey's cholera vaccine prepared with casein hydrolysate, which protected experimental animals more strongly than agar-grown vaccines, and noted with interest that this vaccine was to receive field trial in parallel with standard vaccines in areas where cholera epidemics were expected.

6.1.2. The Group noted that serological response was much higher to a second injection of cholera vaccine than to the first, even when an interval of several months had elapsed between the two. Such had been the case in the vaccination campaigns in Egypt.

6.1.3. The Study Group further noted that :—

(a) Since normal human serum (i.e. the serum of persons unvaccinated with cholera vaccine and not having suffered from cholera) has no appreciable agglutinative action on *V. cholerae*, and

(b) Since the serum of persons once inoculated with cholera vaccine does not seem frequently to show an agglutinative state above 1 : 50, the discovery of a definitely raised agglutination titre for this organism (say 1 : 250—1 : 2,500 or more) in persons who have not been more than once vaccinated, offers strong presumptive evidence that such persons have suffered relatively recent choleraic disease and may allow a proper retrospective diagnosis to be made.

Even where there is a recent history of a relatively recent inoculation with cholera vaccine an agglutination titre above 1 : 500 must raise suspicion of previous infection.

6.1.4. The Study Group recommended that this observation should be confirmed.

6.1.5. The Group, after considering the studies made in Egypt on the reaction after and response to vaccination of infants from the first month to the fifth year of life, noted that the serological response to vaccination was definitely less in infants under two years than in older children. Although at all ages reaction to vaccination was negligible, the Group decided to recommend that the inoculation of children under one year of age should not be required.

6.2. *Sterilization of cholera carriers by means of sulphonamides.*—The Study Group recommended that the use of sulphaguanidine be tried in carriers, both convalescent and contact, with a view to ascertaining its action in shortening the period of vibrio excretion.

6.3. *Bacteriological examination of convalescents and contact carriers in International Quarantine.*—6.3.1. The Study Group, on reconsidering their previous opinion in this respect, decided to recommend that, for purposes of International Quarantine, convalescents and contact carriers be released from restrictive measures after three successive negative bacteriological examinations of their stools or rectal swabs, the second and third examinations being carried out on the fourth and seventh days after the first.

6.3.2. The Study Group recommended that for the purpose of the above measures all passengers on board an *infected* ship or aircraft be considered as contacts.

7. Further international action recommended in respect of cholera.

7.1. The Study Group stressed the desirability of holding its next session in a country where cholera is endemic and where the knowledge and experience of local experts could be made readily available to the Group.

7.2. In view of the very considerable number of problems awaiting investigation by its members, the Group recommended that its next session should be held not earlier than the last quarter of 1949.

7.3. The Study Group further recommended that WHO should make provision for the organization in 1949 of teams of cholera specialists and for their despatch in 1950 to selected endemic areas, with a view to studying and demonstrating in limited districts methods of cholera eradication, on the basis of the results to be expected from the field studies recommended by the Group for 1948-49 of the factors governing cholera endemicity.

MALARIA.

1. Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.

Work on the preparation of suspensions and emulsions of DDT was continued as during the previous year. So far a better method of preparing a suspension than the one developed last year has not been found. Efforts to find a satisfactory explanation for the failure of some samples of DDT to form a suitable suspension have not been successful.

A number of samples of concentrated pastes containing 50 to 90 per cent DDT was received for tests. Unlike the concentrated pastes tried already (about three years ago) in the present samples no mineral oils had been used as solvents. These pastes could be easily diluted with water without any buttering but a small amount of precipitate of different consistency in the various samples quickly formed. If this defect can be removed this formulation will be a great advance on all the others so far tried.

A 25 per cent miscible solution of DDT was prepared with the help of resin soap and Aromax (MKE). In order, however, to prepare such a highly concentrated solution of DDT with MKE as the solvent the solution has to be heated. Although none of the ingredients is highly inflammable the hazard of fire is certainly there. With ordinary soap solution it is possible to prepare a 20 per cent concentrate but this emulsion in the concentrated form is not stable. Two vegetable saponins were tried as emulsifiers, for DDT-Aromax (MKE) solution: (a) soap-nut powder (*chikakai*) prepared from the pods of *Acacia* sp., widely used in South India and (b) powder prepared by drying and milling the pericarp of the common Dhobi-nut (*Rittha*) *sapindus mukoroas*. The former has very poor emulsifying properties, but the latter has given very promising results. Two methods of using the Dhobi-nut powder were tried. One part of *kuttha* powder was found enough to emulsify 100 parts of a 25 per cent solution of DDT in Aromax. The emulsion has, however, to be vigorously shaken at the time of dilution with water, and it takes time before a proper emulsion is formed. A concentrated emulsion can be prepared by vigorously shaking 1 part of a cold watery extract of Dhobi-nut with 100 parts of a 25 per cent solution of DDT in Aromax. Such a concentrated emulsion is of the mayonnaise type and has been found to be stable for a number of days. Even when it creams up to a certain extent, a little shaking is enough to mix it again into a homogeneous emulsion. This concentrate can very easily be diluted with water to contain even 1 to 2 per cent DDT.

Field experiments to test the comparative efficacy of the different preparations as residual sprays against adult mosquitoes were continued at Delhi and in the Nilgiris.

Five villages were selected in Delhi rural area. In each of these villages 4 catching stations were established and, as in the experiments during the previous years, mosquitoes were collected at regular intervals to note the collection per man-hour, as well as the total mosquito population in each. Spleen rates and the parasite rates among children in these villages were also studied towards the end of July. Inner surface of walls and ceilings of all houses and cattle-sheds in 4 of these villages were sprayed each with a different preparation and one was left untreated for comparison. The different preparations used were :—

1. DDT—gelatine suspension (50 per cent).
2. DDT—Aromax emulsion (25 per cent concentration).
3. DDT—solid emulsion (70 per cent concentration).
4. Gammexane—gelatine suspension.

An analysis of the data so far collected shows that under the conditions prevalent in villages around Delhi a dosage of 50 mg. of DDT per square foot reduces the prevalence of mosquitoes by 90 to 92 per cent within 24 hours of spraying. The density, however, begins to rise gradually after 10 days. With gammexane suspension sprayed at the rate of 1 c.c. per sq. ft. of surface (0·1 per cent gamma isomer) the population of mosquitoes comes down almost by 100 per cent in 24 hours and begins to rise gradually as in the case of DDT. Reduction in the number of flies was noticed in all the villages sprayed but the effect was much more marked in the village sprayed with gammexane than in the others.

In the Nilgiris (a) New Valikadavu, about 20 miles from Gudalur was divided into 2 parts which were almost identical as regards mosquito prevalence. Catching stations were selected after careful search for *A. fluviatilis* from all the houses and daily collections of mosquitoes were made from them at regular intervals to note the per man-hour catch as well as the total mosquito population in each. One part was sprayed indoors with DDT-gelatine suspension (1·25 per cent) at the rate of 25 mg. of DDT per sq. ft. and the other with 1·25 per cent of DDT-MKE rosin emulsion (diluted from 35 per cent rosin-soap emulsion). Old Valikadavu village was left untreated for purposes of comparison.

An analysis of the data collected so far shows that under the prevailing conditions at Valikadavu with high relative humidity, provided all indoor surfaces of walls and ceilings are uniformly sprayed—25 mg. of DDT per sq. ft., whether applied as a suspension or emulsion is enough to keep the mosquitoes at a very low level for a period upwards of 12 weeks and control *A. fluviatilis* 100 per cent for this period thus effectively preventing transmission of malaria.

(b) Gudalur was divided into four separate parts, catching stations were selected and collections made from them at regular intervals as in the other experiments. Three of the parts were sprayed each with a different preparation of DDT and the fourth was left untreated for comparison. The different preparations used were :—

DDT—gelatine suspension (50 per cent).
DDT—MKE emulsion (Aromax) (35 per cent).
Geigy malaria spray (50 per cent).

An analysis of the experiments recorded so far shows that 50 per cent Geigy malaria spray applied at the rate of 25 mg. DDT per sq. ft. gives a hundred per cent reduction of all Anophelines for 17 days, while with suspension and emulsions applied at the rate of 50 mg. of DDT per sq. ft. 100 per cent reduction was observed for 28 to 40 days. In the area where Geigy malaria spray was used *A. fluviatilis* was not found for 10 weeks, after which its incidence became 0·3 per man-hour (as compared to 0·5 per cent per man-hour during the pre-spray period). In the area in which DDT suspension or emulsion was used no *fluviatilis* has been collected so far, three months after spraying. Observations are being continued.

The above findings indicate that under the conditions of high atmospheric humidity, DDT applied at the rate of 50 mg. per sq. ft. effectively prevents

transmission of malaria and causes a marked reduction in the number of Anopheline mosquitoes for a period upwards of 3 months. Observations are being continued.

(c) A group of three houses, Marapalam hamlet, about 2 miles from Gudalur was selected and prevalence of *A. fluviatilis* in it was found to be 16·5 per man-hour, and of all Anophelines as 18·5. Out of 33 *A. fluviatilis* dissected, two showed sporozoite infections. This group of houses was sprayed with DDT-gelatine suspension at the rate of 25 mg. of DDT per sq. ft. This produced a hundred per cent reduction of all Anophelines for about 48 days after spraying. No *fluviatilis* have been collected for over three months while the incidence of other Anophelines during July was 2 per man-hour.

By scraping at regular intervals, surfaces which had been sprayed with DDT and dissolving the DDT out of these scrapings, it has been observed that DDT is more lasting in unplastered bamboo huts than on plastered mud-walls.

Observations made on the behaviour of zoophlic Anophelines have shown that a large majority of them are found resting at night on the fencing and the upright pieces used in the construction of the cattle-sheds. DDT film on these structures becomes ineffective sooner than on other parts because of its being either covered by cow dung or rubbed away by the bodies of the cattle in the sheds. After taking a feed a large proportion of these mosquitoes may rest outdoors or may enter human dwellings towards early mornings. If they enter houses towards early morning not many days after the house was sprayed with DDT it takes very little time for the mosquitoes to get agitated and leave the house. If, however, they enter in the later stages, when the DDT film has lost its toxicity to a certain extent, these mosquitoes will be found in the human dwellings. Thus, the occurrence of cattle-feeders in the human dwellings at a time when domestic species like *A. fluviatilis* is absent does not necessarily mean that the latter which feeds on human beings inside the house and stays in it for a long time is more susceptible to DDT than the former *A. fluviatilis* which feeds on human beings inside the house and stays in it for a much longer time than the cattle feeder.

Observations were continued on the bionomics of *A. fluviatilis*. It was found that in the Nilgiris certain houses harbour large numbers of *A. fluviatilis*, while others hardly over 200 yards away were practically free from them. *A. fluviatilis* seems to exhibit what may be termed as "site infestation". This restricted dispersal of *A. fluviatilis* under normal conditions probably accounts for the relative freedom from malaria of residents living even less than half a mile away from the breeding places if other houses are available nearer the breeding place.

Analysis of a very large number of collections ranging over the whole year shows that *A. fluviatilis* seeks dark areas for resting whether they be on the lower or upper half of a wall. It avoids white-washed areas and cobwebs. In houses constructed of split bamboos, unplastered with mud, which are usually fairly well lighted, *A. fluviatilis* is more or less dispersed. In small dark huts with open eaves *A. fluviatilis* were more commonly found on the lower half of the wall.

Larvicides.

Study on the spreading co-efficient of various oils was continued. It was found that the addition of 0·1 per cent of Triton × 100 or Triton × 155 to Malariol 'B' (Burmah Shell) increased the spreading pressure of the oil from 15 to 20

dynes to more than 35 dynes. Turkey red oil when added to Malariol also increases the spreading pressure of the latter. The addition, however, of 0·25 per cent increases the pressure to only about 25 dynes. The extent of spread of a drop of this preparation was studied in the field in natural collections of water and it was found that a drop of Malariol with 0·1 per cent Triton×155 spread over a surface 5 ft. in diameter, Malariol with 0·1 per cent Triton×100 spreads 3½ to 4 ft. diameter, while with 0·25 per cent Turkey red oil about 3 ft. only.

A number of separate breeding places of Anopheline larvæ was selected and the 3 preparations were applied at the rate of 2 c.c. per 10 sq. yards. Analysis of the results showed that with Triton×155, a 100 per cent kill was obtained, with Triton×100, 98 per cent and with Turkey red oil 85 per cent in 24 hours. A team of workers was sent to Kurukshetra to help in controlling the prevalence of flies, fleas and lice in the Central Refugee Camp. Various preparations of DDT and gammexane were tried. It was found that although when DDT emulsions and suspensions were freshly applied, the incidence of flies would go down appreciably, the residual effect of these preparations was not very prolonged. This may possibly have been due to the very low humidity and also to the large amount of dust which was all the time settling down on the sprayed surfaces. Gammexane suspension appeared to be more effective against flies than DDT but large quantities of gammexane were not available for any prolonged tests.

The problem of controlling tick-borne typhus with the help of insecticides was also studied at the request of army authorities. DDT powder did not seem to have very pronounced effort on the ticks and gammexane appeared to be better but the experiments were not very conclusive and could not be continued owing to the paucity of material. Further experiments are being carried out on suggested lines by the army authorities.

2. Enquiry on the control of sandflies *P. argentipes* by spraying with DDT under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta.

Certain outstanding features in regard to the control of sandflies by DDT spraying :—

1. *Breeding places of sandflies.*—

(a) Cow-sheds : (*P. argentipes*)	Inside cow-shed :	Under the feeding troughs in corners, and on floors where the soil is moist.
	On the outside of cow-sheds :	Up to a distance.
(b) Rat-holes : (*P. argentipes*)	In moist places :	None found in rat holes where the soil was dry.
(c) Stumps of banana trees : (*P. minutus*)	In moist soil.	
(d) Dwelling huts : (*P. argentipes*) (*P. papatasii*)	In smaller numbers than in cow-sheds.	

2. *Cow-sheds.*—Average size : 15′×9′×9′.
Bed rooms.—Average size : 16′×14′×12′.

3. *Maximum flight of sandflies :* 50 yds. [From cow-sheds to living quarters as detected by precipitin test (across open ground)].

4. *Strength of DDT solution.*—5 and 2½ per cent solution. Prepared by dissolving pure DDT powder in grade III kerosene in a pestle with a mortar, thereafter exposing the receptable in bright sunshine, the mixture being frequently agitated till no deposit of the powder is found on keeping.

5. *Dosage.*—Three ounces per square foot area has been the average quantity used.

6. *Sprayer.*—Jid Dandy type used.

7. *Parts sprayed.*—(a) On the inner walls—up to a height of 3 ft. from the ground.

(b) Under and around feeding troughs.

(c) In moist places on the floor.

8. *Residual effects in cow-sheds and living huts.*—(a) Sprayed once during June, July or August and some during November, 1947. Effects lasting for 7 to 8 months.

(b) During 1948 : Results of new spraying and re-spraying : Free from sandflies uptil now (4 to 5 months).

9. *Incidence of sandflies.*—Appears in April and the peak gradually rises up to September, then falls gradually. In November to March, their number is very small.

3. Enquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

Paludrine.—Clinical trials with paludrine were given in a series of 170 hospital patients. Eighty among them had *P. falciparum*, 79 *P. vivax*, 5 *P. malariæ*, 4 mixed *P. falciparum* and *vivax* and 2 mixed *P. falciparum* and *malariæ* infections. The cases were divided into 8 groups, 6 having the drug by mouth and 2 by intravenous or intramuscular injections. The dosage used in the former 6 groups and its effect on the temperature and asexual parasites are shown below :—

Schedule of treatment.	Number of cases.	Last day of fever :		Last day of asexual parasites :	
		3rd day or earlier	4th day or beyond	3rd day or earlier	4th day or beyond
A. 0·1 g. in a single dose	11	8(72%)	3(28%)	9(81%)	2(19%)
B. 0·3 g. in a single dose	42	37(88%)	5(12%)	37(88%)	5(12%)
C. 0·1 g. t.d.s. for 4 or 10 days	58	48(83%)	10(19%)	54(93%)	4(7%)
D. 0·2 g. 6 hrly for 4 days	12	8(66%)	4(34%)	8(66%)	4(34%)
E. 0·3 g. b.d. for 10 days	11	9(82%)	2(18%)	10(91%)	1(9%)
F. 0·6 g. or 0·8 g. in a single dose ...	12	8(66%)	4(34%)	11(91%)	1(9%)

The effect of the treatment in all the above 6 groups was that the fever subsided in 74 per cent cases and the asexual parasites disappeared from the peripheral blood in 88 per cent cases by the end of the 3rd day of treatment. There is an indication that 0·3 g. may be regarded as the optimum dose either for a single dose treatment or for repeated daily doses. It is doubtful if a higher daily dose is likely to cut short an attack.

It may be noted that the gametocytes are not affected by this drug. The crescents often persist for long periods in the peripheral blood even up to 61 days after the cessation of treatment.

No side effects were met with in groups A, B and C but toxic reactions were noticed in five patients of group B, one patient of group E and three patients of group F receiving 0·8 g. in a single dose. Gastro-intestinal irritation accompanied by nausea, vomiting, diarrhœa and griping was the commonest untoward symptom and renal irritation occurred in two cases of group D, one having hæmaturia. The administration of the drug was discontinued on the appearance of these toxic symptoms but in a few cases where the reaction was mild the drug was continued.

Early relapse within a month during their stay in the hospital was common with groups A and B the rates being 85·7 and 29 per cent respectively. Only 4 cases of group A and 5 cases of group B were followed up after discharge, 3 of the former and 2 of the latter relapsed (3 parasitic, 2 clinical), while the other 4 were free from fever for 5 to 21 months. Out of 8 patients in group C while in the hospital for over a month, 2 relapsed. One of them with *P. vivax* infection was treated with 0·1 g. t.d.s. for four days. He had a recurrence 17 days after treatment. The other, a case of *falciparum* infection relapsed twice after 9 and 11 days, the initial attack and the first relapse being treated with 0·1 g. t.d.s. for 10 days. The second relapse was treated with a different anti-malarial drug and there was no recurrence for 11 months subsequently. It was possible to follow up 12 cases of group C who had 0·1 g. t.d.s. for 4 days of whom 7 relapsed (5 parasitic, 2 clinical) and 5 were free from attacks for 4 to 20 months. In group D two *vivax* cases relapsed in the hospital 18 and 24 days after treatment and one *vivax* case who was under observation for two and a half months had no further attacks. There has been so far no relapse in group E who were only recently treated. Four patients of group F relapsed within a month after treatment and one *falciparum* case who is under observation for nine months has been free from relapse. Taking all the cases (except group A) who were under observation for over a month 23 out of 58 cases that is about 4·0 per cent relapsed. The blood smears showed homologous parasite in 21 cases and the blood was not examined in the other 2 cases. Early relapses were more common in *falciparum* infection and late relapse in *vivax* infection. Re-infection could not be excluded in at least some of the cases. Some patients are having bi-weekly doses of paludrine after the therapeutic course and they have not been taken into account in the above analysis.

A bi-weekly dose of 0·1 g. of paludrine hydrochloride was given to 20 patients for varying periods after the therapeutic course and no one had a recurrence during the period the drug was administered.

Parenteral therapy.—(a) Paludrine lactate has been tried intramuscularly in single doses varying from 100 mg. to 300 mg. in 13 patients. The injections were painful but the pain and local tenderness usually disappeared within 3 days. On post-mortem examination in a fatal case no signs of necrosis were

noticed at the site of injection. Excepting one case of pernicious malaria with very heavy infection which died within 36 hours of admission, the temperature was normal and the peripheral blood was cleared of asexual parasites by the end of the third day of treatment in most of the cases. In this series three *falciparum* cases relapsed after 3 weeks and one *vivax* case after 6 weeks.

(b) Paludrine acetate was tried intravenously in 11 cases all of whom except 2 had *falciparum* infection. Generally, these patients had either cerebral or gastro-intestinal symptoms. Four were gravely ill. The dosage varied from 25 mg. to 400 mg. and was repeated only in a few cases. The total amount of paludrine received by injection was from 200 mg. to 600 mg. for an individual patient. The number of cases in this series was too small to allow determination of the optimum dosage, but a dosage of 200 mg. to 400 mg. in one injection was usually sufficient to control the temperature and clear the peripheral blood of parasites in 2 to 3 days. One patient admitted in a moribund state and treated in the way stated died. Another with typical cerebral malaria remained unconscious for several days and later developed encephalitis from which, however, he recovered. The injections were well tolerated but two patients had phlebitis; this subsided in a few days.

Chloroquine.—Chloroquine (SN 7618) was tried on 50 hospital patients. Two dosage schemes were employed. Group I: consisting of 0·5 g. of the base for the first dose followed by 0·25 g. after 6 hours and then 0·25 g. on each of the two following days, comprising of a total of 1·25 g. This was tried in 42 cases. Group II: consisting of a single dose of 1·5 g. This was followed in 8 cases. In group I the temperature came down to normal in an average period of 26·8 hours and in group II 24·9 hours. In group I the parasites disappeared in 35 hours and in group II in 25·5 hours. The disease appeared to be controlled more quickly in *vivax* than in *falciparum* infection. None of the above cases had a relapse in the hospital. Out of 20 patients who were followed up after their discharge from the hospital for 1 to 7 months, 20 per cent (2 *falciparum* and 2 *vivax*) relapsed. The gametocytes were apparently unaffected but in those cases in which they were absent when the tretament was started, they frequently failed to appear. Mild and transitory toxic symptoms were noticed in 5 cases (10 per cent). Chloroquine thus seems to be a more effective drug in terminating an attack than quinine, mepacrine or paludrine.

CAM-AQI.—CAM-AQI or Camoquin, a quinoline derivative was tried on 55 patients. Two dosage schedules were employed.

Group I.—0·1 g. twice daily for 3 days tried in 27 cases and

Group II.—a single dose of 0·5 g. tried in 28 cases. Out of 55 patients thus treated, 23 had *falciparum*, 23 *vivax*, 3 *malariæ* and 6 mixed *falciparum* and *vivax* infections. In group I the temperature came down to normal in an average period of 30 hours, and in group II in 22 hours. The asexual parasites disappeared from the peripheral blood in an average period of about 24 hours with any of the two methods of treatment. The drug was found to be equally effective against all the three forms of plasmodial parasites.

For the purpose of studying the relapse rate 26 cases were observed for 1 to 8 months. During this period 9 cases, i.e. 34·6 per cent relapsed (8 parasitic, 1 clinical). Out of these 9 cases only one case of *falciparum* infection had an early relapse in the hospital within 20 days. The other 8 cases had *vivax* infection either alone or with *P. falciparum*, and in 7 of these *P. vivax* alone was found during the first relapse which occurred after a month or at a later

date. Relapse was more frequent in group I than in group II. The drug did not seem to have any marked effect on the gametocytes. The *vivax* and *malariæ* gemetocytes disappeared within 5 days, while crescents persisted up to 26 days after treatment. The drug was well tolerated and no untoward reaction was noticed. The therapeutic response with Cam-aqi was noticed to be more rapid than quinine, mepacrine or paludrine and might be compared to that of chloroquine used in a much higher dose.

Field study on chemoprophylaxis of malaria.—The experimental work for the control of malaria in the rural areas of Bengal with weekly or bi-weekly administration of the newer synthetic anti-malarials has been commenced. The site selected is a hyper-endemic malarious area in the Hooghly District about 40 miles from Calcutta and comprises of 9 villages with a radius of about three miles. The inhabitants are mostly cultivators and labourers. Little medical facilities are available there. The initial survey carried out in the month of May 1948 included estimation of the spleen and parasite index of the entire group together with detailed examination of a large section of the population. An attempt is being made to find out the influence of general health and social conditions on malaria.

Altogether 1,458 persons have been examined of whom 28·3 per cent are children below 10 years of age. The population has been divided into 4 groups of 250 to 300 persons in each, 3 for chemo-prophylactic investigations and one for control. The prophylactic doses for adults have been arranged as follows :—

Group 1	...	Paludrine hydrochloride	...	0·1 g. twice a week.
Group 2	...	Paludrine hydrochloride	...	0·3 g. once a week.
Group 3	...	Chloroquine	...	0·25 g. once a week.
Group 4	...	Control.		

The regular administration of the drug to the first three groups has been commenced from the 1st week of July 1948 and the control group is receiving quinine sulphate only in case of fever with positive blood smear. The Senior Field Assistant appointed for this work has been stationed at Kantalgoria. He is in-charge of distributing the drug and is responsible for seeing that the medicine is taken in his presence. The blood of every case reported to be ill is examined and if parasites are detected a single therapeutic dose of the drug prescribed for prophylactic purposes in that group is administered. The administration of the drug will be continued throughout the transmission season without any anti-mosquito measures and parasite rate determined at intervals of 2 months. A more detailed examination including spleen and parasite rate will be carried out at the end of the prophylactic regime.

4. Enquiry on the chemotherapy of malaria under Dr. K. Ganapathi at the Haffkine Institute, Bombay.

Synthesis of compounds with possible anti-malarial activity.—Two types of compounds are being synthesized with the above object in view. One type comprises compounds of the thiazole series with a *para* chloro-phenyl-amino group attached to the thiazole ring which carries various substituents characterizing the well-known anti-malarials. About twenty such compounds have been prepared ; they are easier and cheaper to prepare than chloroquin and the idea is to discover a drug that can possess comparable anti-malarial activity but far cheaper to produce. The compounds of the second type comprise 4-substituted quinolines of which about twenty compounds have been synthesized.

In this case the object is to prepare a 4-substituted quinoline compound showing activity also against the exo-erythrocytic forms of the plasmodia. Such a compound should be more valuable than chloroquin and could be expected to give radical cures and serve as a true casual prophylactic against *vivax* malaria. Attempts to prepare compounds with a bi-guanide side chain at the position 4 of quinoline ring by customary methods are offering great deal of synthetic difficulties.

Testing of the anti-malarials activity of the compounds.—Considerable difficulty is being experienced in procuring a steady supply of chicks required for the testing work. No regular dealer or the poultry-breeder including the Bombay Government Poultry Farm has been able to give a regular supply of chicks. Attempts are, therefore, being made to build a poultry farm at the Institute itself as early as possible. In the meanwhile the staff employed are getting training in the technique of the testing methods to be employed. A colony of *Aëdes aegypti* has been raised.

List of papers published.

CHAUDHURI, R. N., RAI CHAUDHURI, M. N., and CHAKRAVARTY, N. K.—Chloroquine (SN 7618) in Malaria (*Ind. Jour. Malariol.*, June 1948).

CHAUDHURI, R. N., and CHAKRAVARTY, N. K.—Clinical Trials of CAM-AQI in Malaria. (*Ibid.*)

CHAUDHURI, R. N., and CHAKRAVARTI, H.—Intravenous Paludrine. (*Brit. Med. Jour.*)

CHAUDHURI, R. N.—Treatment of Malaria. (*Ind. Med. Gaz.*, May 1948).

Report of the meeting of the Malaria Advisory Committee held in the Senate House, Madras, on the 1st December, 1948.

PRESENT

Lieut.-Colonel Jaswant Singh. (*Chairman*).
Dr. B. Ananthaswamy Rao.
Dr. A. C. Banerjea.
Bt.-Colonel Sir Ram Nath Chopra.
Dr. M. O. T. Iyengar.
Dr. K. V. Krishnan.
Dr. P. Kumaran Nair.
Dr. D. N. Roy.
Dr. D. K. Viswanathan.
Dr. S. K. Viswanathan.
Dr. I. M. Puri.
Dr. S. P. Ramakrishnan. } (*Secretaries*).

The following were also present :—

Dr. A. K. Adhikari.
Colonel S. L. Bhatia.
Dr. R. Bhasker Rao.
Major-General A. C. Chatterji.
Dr. R. N. Chaudhuri.
Dr. B. C. Das Gupta.
Lieut.-Colonel A. N. Duggal.
Dr. A. Gilroy.
Lieut.-Colonel B. N. Hajra.
Dr. C. S. P. Hamilton.
Dr. A. R. Mehta.
Dr. J. M. Mungavin.
Major N. G. S. Raghavan.
Dr. S. Rajendram.
Dr. T. Ramachandra Rao.
Dr. J. C. Ray.
Major R. Subramanian.
Dr. K. V. Venkatraman.

PROCEEDINGS.

The reports on enquiries carried out during the current year and proposals or 1949-50 were considered.

A. EXISTING ENQUIRIES.

1. *Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.*—A large number of samples of concentrated pastes containing 50 to 90 per cent DDT were tested. Notable feature in the preparation of these pastes was that no mineral oil had been used as solvent of DDT. In diluting these pastes with water a certain amount of heavy precipitate settled down. If this defect can be overcome this formulation would prove a great advance on all the other formulæ so far tried.

A miscible solution of DDT (35 per cent) was prepared by dissolving it in hot medium kerosene extract and emulsifying the solution with rosin soap. Although none of the ingredients is highly inflammable, hazard of fire is certainly there. A 20 per cent concentrate can be prepared with ordinary soap but this emulsion concentrate is not stable. Two vegetable saponins: (a) soap-nut (*Shikakai* powder and (b) common dhobi-nut (*Ritha*) powder, were also tried as emulsifiers for DDT-MKE solution. The latter proved superior to the former. One part of *Ritha* powder was found sufficient to emulsify 100 parts of 25 per cent DDT-aromax solution. At the time of dilution with water the emulsion had to be shaken vigorously. A mayonaise type of concentrated emulsion was also prepared by vigorously shaking 1 part of cold aqueous extract of dhobi-nuts with 100 parts of a 25 per cent solution of DDT in MKE. It remained stable for some weeks and could be easily diluted with water to contain 1 to 2 per cent DDT.

For testing the comparative efficacy of these preparations, five villages were selected in Delhi rural area. Mosquitoes were collected at regular intervals from selected catching stations and the collections per man-hour recorded. Spleen and parasite rates amongst children were also determined. Four of these villages were sprayed, while the fifth was left untreated for purposes of comparison. The different preparations used were :—

1. DDT-gelatin suspension (50 per cent concentrate).
2. DDT-MKE emulsion (25 per cent concentrate).
3. DDT-paste (70 per cent).
4. Gammexane-gelatin suspension.

An analysis of the data showed that under conditions prevailing in villages around Delhi a dosage of 50 mg. of DDT per sq. ft. reduced the density of mosquitoes by 90 to 92 per cent within 24 hours of spraying. The number of mosquitoes began to rise gradually after the first 10 days. With gammexane suspension sprayed at the rate of 1 c.c. per sq. ft. (0·1 per cent gamma-isomer) the mosquito population was reduced by almost 100 per cent in 24 hours and after 10 days the number of mosquitoes began to rise gradually. Reduction in the number of flies in all the sprayed villages was more marked in the villages sprayed with gammexane.

Three villages were selected in the Nilgiris. One village, about 20 miles from Gudalur, was divided into two parts, almost identical as regards mosquito prevalence. One part was sprayed indoors with DDT-gelatin suspension (1·25 per cent) at the rate of 25 mg. DDT per sq. ft. and the other with 1·25 per cent DDT-MKE-rosin emulsion (diluted from 35 per cent rosin-soap-DDT emulsion). Another village was left untreated for comparison. An analysis of the data showed that under the prevailing conditions with high relative humidity 25 mg. of DDT per sq. ft. applied in the form of suspension or emulsion kept the mosquitoes at a very low level for a period upwards of 12 weeks and successfully prevented the transmission of malaria.

The village, Gudalur, was divided into 4 parts, three of which were sprayed with different preparations—DDT-gum acacia-gelatine suspension (50 per cent) ; DDT-MKE emulsion (35 per cent) and Geigy malaria spray (50 per cent) and the fourth was left untreated.

A group of three houses, Marapalam hamlet, about 2 miles from Gudalur was also selected and prevalence of *A. fluviatilis* recorded. This group of houses was sprayed with DDT-gelatine suspension at the rate of 25 mg. of DDT per sq. ft.

Analysis of the data indicated that under conditions of high atmospheric humidity DDT applied at the rate of 50 mg. per sq. ft. effectively prevented transmission of malaria by causing a marked reduction in the number of Anopheline mosquitoes for a period upwards of three months. It was also observed that the effect of DDT was more lasting in unplastered bamboo huts than on plastered mud walls.

In order to find out reasons for the apparent greater resistance of certain species of mosquitoes to DDT deposits, observations were made on the behaviour of zoophilic Anophelines in cattle-sheds. It was found that a large majority rested at night on the fencing and the upright pieces used in the construction of the cattle-sheds. DDT film on these structures became ineffective sooner than on other parts, because of its being either covered by cow-dung or rubbed away by the bodies of the cattle. After a blood feed a large proportion of these mosquitoes rested outdoors or entered human dwellings towards early mornings. Thus, the occurrence of cattle-feeders in the human dwellings at a time when domestic species like *A. fluviatilis* is absent does not necessarily mean that the latter which feeds on human beings inside the house and stays in it for a long time is more susceptible to DDT than the former.

Observations on the bionomics of *A. fluviatilis* and study on the spreading coefficient of various oils as larvicides were continued. The control of flies, fleas and lice in the refugee camps in Kurukshetra was organized and various preparations of DDT and gammexane were tried.

Proposals for 1949-50.

(a) Systematic testing of insecticidal sprays, repellents, larvicides and spraying apparatus under field conditions against various vector species.

(b) To study the bionomics of different vector species in different parts of India.

2. *Enquiry on the control of sandflies P. argentipes by spraying with DDT under Dr. D. N. Roy, School of Tropical Medicine, Calcutta.*—It was found that sandflies breed from April to September in moist places under the feeding troughs and on floors in cow-sheds, in rat-holes, stumps of banana trees and to a lesser extent in dwelling huts. Inner walls of cattle-sheds and living rooms up to a height of three feet from the ground, were sprayed with 5 and 2·5 per cent DDT solution in III grade kerosene. The residual effect of DDT was observed to last for 7 to 8 months.

3. *Malaria enquiry under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta.*—Following regimes of treatment with oral administration of paludrine were tried out. (1) A single dose of 100 mg. in 11 cases, (2) a single dose of 300 mg. in 42 cases, (3) 100 mg. thrice daily for 4 to 10 days in 58 cases, (4) 200 mg. 6-hourly for 4 days in 12 cases, (5) 300 mg. twice daily for 10 days in 11 cases and (6) 600 mg. to 800 mg. in a single dose in 12 cases. In all these series it was found that within 3 days fever subsided in 74 per cent of the cases and asexual parasites disappeared from the peripheral blood in 88 per cent of the cases. In heavier dosage groups toxic symptoms of gastro-intestinal and renal organs were observed in a few cases. Except in group 5, relapses occurred within very short periods.

Intramuscular injections of paludrine lactate was tried out in a single dose varying from 100 mg. to 300 mg. in 13 patients. The injections were painful but the pain and local tenderness disappeared within a days few. The temperature became normal and peripheral blood free of asexual parasites within 3 days of treatment. Relapses occurred within 3 to 6 weeks in both M.T. and B.T. cases treated.

Paludrine acetate was administered intravenously in 11 cases suffering from malignant malaria of cerebral and gastro-intestinal types. A dosage of 200 to 400 mg. was found sufficient to control the temperature and clear the peripheral blood of parasites within 2 to 3 days. One death occurred in a moribund case and another developed encephalitis but recovered later. Two cases developed transient phlebitis. The injections were on the whole well tolerated.

Similar experiments were carried out with chloroquine on 50 hospital cases following 2 regimes of treatment, (1) 0·5 gm. followed by 0·25 gm. six hours later and 0·25 gm. per day for 2 days (total 1·25 gm.) in 42 cases and (2) a single dose of 1·5 gm. in 8 cases. In these doses, the temperature was controlled in an average period of about 26 hours and parasites disappeared within the first 24 to 48 hours. This compound in the dosage given appeared to be more effective in terminating the attack of malaria earlier than quinine, mepacrine or paludrine. Toxic symptoms of mild transient nature were noticed in a few cases.

Another drug, CAM-AQI, was tried on 55 patients—0·5 gm. twice daily for 3 days was administered to 27 cases and 0·5 gm. in a single dose to 28 cases. In the first group, the temperature returned to normal in an average period of 30 hours and in the second in 22 hours. The drug was well tolerated and no toxic symptoms were observed. The drug showed no superiority in so far as relapse rate was concerned.

Field studies on chemo-prophylaxis in selected villages in an hyperendemic area in Hooghly district (West Bengal) were being continued.

Proposals for 1949-50.

Studies on chemotherapy and chemo-prophylaxis of malaria.

4. *Chemotherapy of malaria under Dr. K. Ganapathi, Haffkine Institute, Bombay.*—Two types of compounds have been synthesized and tested. One type comprises thiazole series with a *para* chloro-phyenyl-amino group attached to the thiazole ring which carried various substituents characterizing the well-known anti-malarials. The second type comprised 4-substituted quinolines. Attempts to prepare compounds with a biguanide side chain at position 4 of quinoline ring by customary methods were not very successful.

Proposals for 1949-50.

(1) Synthesis of compounds with possible anti-malarial activity particularly against the exo-erythrocytic forms of plasmodia, and

(2) Assessment of their activity against *P. gallinaceum* in chicks.

B. Proposals for new enquiries.

1. *Enquiry on the control of kala-azar by controlling sandflies* P. argentipes *under Dr. D. N. Roy, School of Tropical Medicine, Calcutta.*—It is proposed to control kala-azar by killing sandflies with DDT-spraying in a group of villages in West Bengal.

2. *Enquiry on sex determination in* Chrysomyia rufifacies *under Dr. D. N. Roy, School of Tropical Medicine, Calcutta.*—A cytological study on the chromosomes to determine sex phenomenon in *Chrysomia rufifacies* is intended.

3. *Studies on the insecticidal and larvicidal properties of Indian flowers belong to the* N. O. Compositæ *under Dr. S. M. Ghosh, School of Tropical Medicine, Calcutta.*—It is intended to study the effect of the extracts of pyrethrum flowers on insects and their larvæ.

4. *Enquiry on complement-fixation test in malaria for the assessment of anti-malarial drugs under Dr. J. C. Roy, Indian Institute of Medical Research, Calcutta.*—To investigate whether complement-fixation test may be introduced for testing the comparative merits of anti-malarial drugs.

The Committee made the following recommendations :—

A. Existing enquiries.

(1) Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.

(2) Enquiry on the control of sandflies, *P. argentipes*, by spraying with DDT under Dr. D. N. Roy, School of Tropical Medicine, Calcutta.

(3) Enquiry on malaria under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta.

(4) Enquiry on the chemotherapy of malaria under Dr. K. Ganapathi, Haffkine Institute, Bombay.

The Committee approved the proposals put forward for the continuance of enquiries Nos. 1, 3 and 4 and noted that it was not proposed to continue enquiry No. 2.

B. Proposals for new enquiries.

I. The Committee felt that studies on the control of kala-azar by controlling sandflies could be included as one of the functions of the insecticide and mosquito repellent enquiry. After some discussion the proposal for this new enquiry was withdrawn.

The Committee recommended the proposals on complement-fixation test for the assessment of anti-malarial drugs under Dr. J. C. Ray but did not approve the proposals put forward in (1) enquiry on sex determination in *Chrysomyia rufifacies* under Dr. D. N. Roy and (2) studies on the insecticidal and larvicidal properties of Indian flowers belonging to the *N.O. Compositæ* under Dr. S. M. Ghosh.

II. The Committee discussed the validity of malaria morbidity statistics as recorded in out-patient departments by medical officers in charge of dispensaries in different levels of malarial endemicity and Dr. D. K. Viswanathan agreed to submit formal proposals in due course.

III. The Committee appreciated the views expressed by Col. S. L. Bhatia, Surgeon-General with the Government of Madras, on the desirability of undertaking large-scale clinical researches in hospital cases side by side with malaria in monkeys and birds at major centres like the Malaria Institute of India where other aspects are being studied.

NUTRITION.

1. Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, Coonoor.

I. Studies on the vitamins of the B complex.

(a) *Excretion of thiamine and riboflavin by Indian adults.*—This investigation has been undertaken with a view to arriving at a correct estimate of the requirements of thiamine and riboflavin by the Indian adults. The investigation includes the estimation of 24-hour excretion of thiamine and riboflavin, one-hour fasting excretion, determination of the blood levels and response to load test. So far, a number of observations on the 24-hour excretions of thiamine and riboflavin on the animal attendants and other workers at the Nutrition Research Laboratories have been made, both on unrestricted diets and on restricted diets. The 24-hour excretion of thiamine has been found to vary between 66 µg. and 1,200 µg. The day-to-day variation in the same subject is much less than these figures would suggest. A few one-hour excretion tests have also been carried out where the amount of thiamine excreted was found to vary between 0.062 µg. and 0·12 µg. thiamine per millilitre. Similar observations have been made with regard to riboflavin excretion. The 24-hour excretion of riboflavin as found in 50 determinations varied from 340 µg. to 4,401 µg. Individual variations are much less in magnitude, as also is the variation in the fasting one-hour sample expressed as riboflavin per ml. of urine, the minimum and maximum being 0·14 µg. to 1·4 µg. per ml. respectively. The observations are being continued on a larger number of subjects.

(b) *Biosynthesis of nicotinic acid from tryptophane by germinating seeds.*—Earlier work in the United States has shown that tryptophane can replace to a certain extent nicotinic acid in the body. The mechanism of conversion of tryptophane to nicotinic acid is being further investigated. There is reason to believe that in this transformation, the intestinal bacteria do not play an important rôle but that it is the tissues which bring about the synthesis. The actual investigation at the present moment deals with the conversion of tryptophane by germinating cereals and legumes. These latter are allowed to germinate under sterile conditions in presence of, or in absence of tryptophane. It was found that certain seeds germinating in tryptophane solution showed a greater amount of nicotinic acid than those which were allowed to germinate in the presence of sterile distilled water. Further experiments designed to study the conditions of germination and its effect on conversion of tryptophane into nicotinic acid are in progress.

(c) *Pantothenic acid deficiency and acetylation mechanism.*—It has recently been shown that co-enzyme A contains large amounts of pantothenic acid and that this enzyme takes part in the acetylation mechanism in the body. The acetylation of para-amino-benzoic acid in rats has been reported to depend on an adequate intake of pantothenic acid. It is intended to confirm the above observation in human cases of pantothenic-acid deficiency coming to the clinic. A few cases of burning-feet syndrome have been seen at the clinic and a study of the capacity of such patients to acetylate a given dose of para-amino-benzoic acid before and after treatment is being undertaken.

(d) *Pyridoxine and fatty acid metabolism.*—The deficiency of pyridoxine in rats causes a peculiar kind of dermatitis which in many ways is analogous

to that found in deficiency of essential fatty acids. There is evidence to show that the dermatitis caused by either of the two above-mentioned nutrients can be influenced favourably by dosage with not only the missing nutrient but by the other as well. When the two are administered together, dermatitis is cured earlier than when one is exhibited alone indicating that there is probably a correlation between the metabolism of fatty acid and pyridoxine. This possibility is being investigated in rats which are subjected to pure pyridoxine and essential fatty acid deficiency as well as a mixed deficiency of the two. The study of serum lipoids and the lipoids of liver and kidney is expected to throw some light on the inter relationship between pyridoxine and the essential fatty acids.

(c) *Folic acid and storage of vitamin A in liver.*—Recently some reports have appeared to the effect that in rats the storage of vitamin A in liver depended upon an adequate supply of folic acid. Experiments to test this were undertaken and it was observed that when vitamin A deficient rats were given (a) vitamin A alone or (b) vitamin A with folic acid, greater storage of vitamin A in the liver was found in the rats of the latter group. This interesting observation is being followed up to elucidate the action of folic acid in promoting vitamin A storage.

II. Studies on vitamins A and D.

(a) *Conversion of carotene into vitamin A in the intestine of the albino rat.*—The available evidence till now did not permit definite conclusions to be drawn about the site of conversion of carotene to vitamin A in the body. Some experiments undertaken in this laboratory have indicated that this conversion takes place in the lumen of the small intestine. Vitamin A deficient rats were fed on a fasting stomach 4·375 mg. of carotene and vitamin A in the intestinal contents, the wall of the small intestines, the blood and the liver was estimated in a number of rats at different intervals. From the results obtained, it is believed that the conversion of carotene does take place in the lumen of the small intestines. Further study of the mechanism involved is in progress.

(b) *Mode of action of vitamin D.*—The most important manifestation of vitamin D action, i.e. its influence on calcification of bones is being investigated. The action could be a local one at the site of calcification or might be one of the manifestations of its general action. With a view to deciding between the two, *in vitro* calcification experiments have been undertaken. Bone slices of rachitic rats are immersed in calcifying solutions and the extent and the intensity of calcification after incubation at 37° C. for 21 hours are observed. A basal solution containing the proportions of calcium, inorganic phosphorus and ester phosphorus of 9 : 5 : 1 has been found most suitable as the control calcifying solution. The influence of the addition of protein, vitamin D in propylene glycol or alcoholic solution and in emulsion has been tested. In no case, any evidence of a local action of vitamin D on the calcification of bone was found. Sheep serum in which the rachitic bones were found to calcify was incubated with vitamin D solution. No further increase in the extent and intensity of calcification was found in these fluids as compared to that taking place in control solutions. Further work on the influence of a large dose of vitamin D given to animals and the effect of calcification in serum drawn from such animals is in progress.

(c) *The anti-rachitic activity of synthetic compounds.*—Three derivatives of cinnamylidene cyclohexane synthesized by Professor Bhide of S. P. College, Poona were tested for their anti-rachitic activity in rats kept on rachitogenic diets. The bone-ash content and the examination of the epiphyseal cartilage after staining showed that none of these compounds possessed any anti-rachitic activity.

III. Studies on hydrogenated oils.

(a) *Human metabolism experiments.*—These experiments were undertaken as a part of the co-ordinated investigation on the nutritive value of hydrogenated oils sponsored by the Vanaspati Research Advisory Committee of the Technical Panel, Ministry of Food, Government of India. Six adult male volunteers were placed on a poor South Indian diet of the following composition : milled rice : 18·6 oz., pulse (*Cajanus indicus*) : 0·7 oz., non-leafy vegetables : 2·0 oz., leafy vegetables : 0·5 oz., and whole-milk powder : 0·2 oz. (re-constituted into whole milk). This diet was supplemented with 68 g. of fat under investigation per volunteer per day. The fats investigated were (i) crude ground-nut oil, (ii) refined ground-nut oil, (iii) hydrogenated ground-nut oil of melting point 37°C., (iv) hydrogenated ground-nut oil of melting point 41°C. and (v) milk ghee. On each fat supplement there was a preliminary period of five days, the collection of urine and fæces being done on the subsequent five days. The amount of fat excreted in fæces and the amounts of nitrogen, calcium and phosphorus excreted in fæces and urine on the respective diets are given in Table I :—

Table I.

Digestibility and metabolic data.

Poor Madrassi diet : Averages of six subjects.

Particulars.	Ground-nut oil.		Vanaspati.		Cow ghee.
	Crude.	Refined.	m.p. 37°C.	m.p. 41°C.	
Fæcal excretion of fat at constant intake of 68 g. per day in g.	4·06	4·35	4·27	6·88	4·07
Apparent digestibility, per cent ...	94·03	93·60	93·73	89·89	94·02
Nitrogen balance on daily intake of 9·36 g. N in g. ...	+2·71	+2·29	+2·64	+2·43	+2·76
Phosphorus balance on daily intake of 840 mg. P in mg. ...	+127	+102	+142	+52	+182
Calcium balance on daily intake of 604 mg. Ca in mg.	+70	+37	+22	−42	+65

IV. Studies on erythrocyte phosphatase.

The characteristics of the alkaline phosphatase isolated from the erythrocytes of different species of animals have been further studied. The enzyme is activable by Mg^{++} and is inhibited by $ZnSO_4$, NaF and NaCN. There seems to be a species difference in the action of these inhibitors. The hydrolytic activity of sheep r.b.c. alkaline phosphatase was tried against the following esters, these are mentioned in the decreasing order of activity shown against them by the enzyme: sodium phenyl-phosphate, sodium phospho-mannonate, sodium pyro-phosphate, sodium mannose mono-phosphate and sodium glycero-phosphate. In rats rendered rachitic there was a considerable decrease in the plasma-alkaline phosphatase but no appreciable change in the activity of erythrocyte alkaline phosphatase was observed. This observation points to the conclusion that erythrocyte alkaline phosphatase cannot be considered a source of plasma alkaline phosphatase.

V. Metabolic disorders of the liver.

The comparative low biological value of proteins and varying degree of deficiency of vitamins and minerals in the poor Indian diets might be considered as potential causes in the metabolic diseases of the liver. The rôle of methionine and cystine, choline, biotin and inositol in cirrhosis of the liver with or without fatty infiltration has been established by work elsewhere. Investigations were, therefore, undertaken to study the effect of typically poor Indian dietaries on the production of liver disorders in the albino rat.

Groups of rats are being fed on (a) Diet I: rice 72 parts, milk 7·14, pulse (*Cajanus indicus*) 3·57, amaranth 7·14, raw plantain 7·14, gingelly oil 1·79 and NaCl 0·7 parts, (b) Diet II: whole wheat 55 and maize starch 17 replacing rice in diet I, (c) Diet III: whole wheat replacing rice in diet I and (d) the Colony Stock Diet consisting of whole-wheat chapatti, sprouted gram (*Cicer arietinum*), milk and mixture of carrots and cabbages.

Four animals from each group have been sacrificed at intervals of 2, 4, 6, 9 months. Before killing they are placed in the metabolic cages and nitrogen and sulphur metabolism is studied. The livers of these animals have been examined for their protein, crude fatty acid, carotene, vitamin A, thiamine and riboflavin content.

Most of these examinations have revealed no significant change in the chemical composition of the liver with the exception of the crude fatty acid content. In spite of the very low fat content of the experimental diets, the livers of experimental animals have shown the crude fatty acid content as high as 10 or more on certain occasions. The rise in crude fatty acid is more rapid in rice group than in the wheat group and is followed by a fall as shown in Table II :—

Table II.

Liver weights and crude fatty acid content of albino rats on poor Indian diets.

Diet.	At 2 months		At 4 months		At 6 months		At 9 months	
	A	B	A	B	A	B	A	B
Diet I ...	5·25	7·38	3·68	11·41	3·68	6·55	3·56	5·62
Diet II ...	3·27	7·63	4·09	7·02	3·31	10·0	...	...
Diet III ...	3·30	6·71	3·65	7·19	3·20	9·14	3·32	6·04
Stock diet ...	4·56	3·80	4·01	3·40	3·44	4·86	3·62	5·46

$A = \frac{\text{Liver-weight}}{\text{Body-weight}} \times 100.$ B = Per cent crude fatty acids on liver.

During the 9 month period, the crude fatty acid (c.f.a.) content of control animals has risen from 3·8 per cent to 5·46 per cent only. The macroscopic appearance of livers having high c.f.a. content has not indicated any gross abnormality. Considering that the damaging effects of these diets which are not totally deficient in essential nutrients and not as grossly imbalanced as some used by most other workers in similar experiments, it is suggested that longer time has to elapse before any change in the structure or function of the liver can become manifest.

VI. Experimental lathyrism.

Experiments to induce toxic effects of feeding with *Lathyrus sativus* in dogs and monkeys have been reported last year. Out of two dogs kept on the diet containing 80 per cent *Lathyrus sativus*, only one developed stiffness of hind limbs and deviation of the lower jaw and the tongue to the left. This animal was later killed and the brain, spinal cord and pieces of sciatic and anterior and posterior tibial nerves were removed at post-mortem. These were fixed in formalin. The specimens are now being examined by Major L. Krainer at the Central Military Pathological Laboratory, Poona, and his report is awaited. No macroscopic abnormalities were noted in other organs examined post-mortem.

Monkeys were fed for over nine months on diets containing 88 to 94 per cent *Lathyrus sativus* with or without husk but none showed any signs of disease. The experiment was, therefore, discontinued.

A second experiment on puppies was started in June 1948. Six puppies from the same litter are being used, four of them have been kept on the *Lathyrus* diet and two on control diet. The composition of the diets is as follows :—

The control diet contains Bengal gram (without husk) 80 parts, skim-milk powder 11, gingelly oil 5, calcium phosphate 2, and sodium chloride 2. In the experimental diet, Bengal gram is replaced by an equal quantity of *Lathyrus sativus* (without husk).

The pups were started on the diets at ten weeks of age. The amounts of Bengal gram and *Lathyrus* pulse were gradually increased in stages replacing rice which was originally included in the diet. The pups have now been for two months on diets containing 80 per cent Bengal gram and *Lathyrus sativus* respectively. One of the animals of the experimental group is already exhibiting stiffness of the hind limbs which has markedly affected its stance, gait and movement in general. It is expected that other dogs too, in course of time, would show similar symptoms. The fact that one dog has already been affected in the same manner as the other dog last year indicates a definite possibility that dog might prove to be a useful animal for the experimental study of the disease.

VII. Clinical investigation.

The Nutrition Clinic established in August 1947 within the premises of the Nutrition Research Laboratories has been functioning satisfactorily. During the last year, 219 cases of nutritional disorders have been examined, investigated and treated at the clinic. The distribution of cases according to the predominant deficiencies is as follows :—

	In-patient.	Out-patient.
Vitamin A deficiency	15	29
Thiamine deficiency	nil	5
Riboflavin deficiency	3	29
Vitamin B Complex deficiency ...	12	15
Vitamin C deficiency	nil	1
Vitamin D deficiency	nil	2
Anæmias	26	9
Deficiency œdema	6	15
General malnutrition including marasmus	1	25
Miscellaneous cases	3	23
Total ...	66	153

The above figures will make it clear that certain deficiencies were more common than others. The wealth of material at the clinic has been utilized for the study of the following problems :—

(a) *Infantile pellagra.*—During the last year, ten cases of pellagra in infants and children between six months and three years of age have been seen. The characteristic features of the disease were diarrhœa, œdema, dermatoses and enlargement of the liver. Associated with these were found symptoms of riboflavin deficiency and in some cases, the picture was complicated by infantile beri-beri. Treatment with nicotinic acid with or without riboflavin and thiamine has proved disappointing. Although the skin lesions markedly and diarrhœa to a certain extent responded to nicotinic-acid therapy, the disease terminated fatally. The syndrome appears to be similar to the one described in South and East Africa by Trowell and his co-workers and also by Gillman and Gillman and called by them Kwashiorkor or malignant malnutrition. It is intended to compare the therapeutic effects of crude liver extracts, desiccated stomach and vitamins of the B complex severally and in combination on the new batch of cases.

(b) *Nutritional diarrhœa in vitamin A deficiency.*—Diarrhœa of a watery type associated with the ocular involvement characteristic of vitamin A deficiency was the chief complaint of the cases under this category. Twenty such cases between the age of 2½ years and 9 years have been investigated and treated with pure vitamin A concentrates. The diarrhœa was controlled within 48 hours and the improvement was maintained throughout the period of observation. Comparative trials with bismuth carbonate and kaolin or with sulphaguanidine were made but results were not satisfactory. The microscopic and cultural examination of the fæces did not reveal the presence of any pathogenic organism responsible for the diarrhœa. There is reason to believe, therefore, that severe diarrhœa can be one of the acute manifestations of vitamin A deficiency.

(c) *Nutritional macrocytic anæmia.*—Out of 35 anæmia patients, twelve turned out on investigation to be cases of macrocytic anæmia. These were fully investigated and treated with folic acid ; detailed information for nine of them (including one suffering from sprue syndrome) is available. Five patients were male and four female between 22 and 55 years of age. The range of initial and final values for blood are given below :—

	Initial.		Final.	
	Min.	Max.	Min.	Max.
R.b.c., million /c.mm. ...	0·96	2·77	3·0	4·86
Hæmoglobin, g. per cent ...	5·0	11·0	11·6	14·8
Packed cell volume	...	...	...	...
M.c.v., cuμ	112·7	312·5	82·3	133·3
M.c.h.c., per cent	16·7	37·14	27·7	39·3
Reticulocytes, peak reached in ...	...	...	5 to 15 days.	
Reticulocytes per cent ...	...	...	8 to 18 per cent.	
Days of treatment required to reach 3 mill. /c.mm. r.b.c.			12 to 34.	

(d) *Ætiology of phrynoderma.*—Dr. Gopalan's work had shown that phrynoderma could be cured by treatment with linseed oil and yeast, thus indicating that the disease may not be due to vitamin A deficiency. Further work has been undertaken for the purposes of confirming these observations and to find evidence for the suggestion that phrynoderma results from a deficiency of or a disturbance in fatty acid metabolism. Cases of phrynoderma are being treated with (a) vitamin A concentrates and (b) shark-liver oil from which vitamin A had been destroyed by æration. Response to the latter treatment has been favourable where vitamin A concentrates had failed. The analyses of the serum lipoids of phrynoderma cases shows that the unsaturation of the serum fatty acids is lower than that observed in non-phrynoderma patients of comparable age. Further work is in progress to elucidate the therapeutically active fatty acid fraction, the effect of one or more members of the vitamin B complex on the cure and the time taken for the return to normal of the serum lipoid composition.

VIII. Field studies.

(a) *Hæmatological standards.*—With a view to studying the changes in the blood at an altitude of 6,000 feet above sea-level and also to establishing the normal standards for the local population, a complete hæmatological investigation was carried out among the troops of the Madras Regimental Centre, Wellington. Such of the troops as had stayed there continuously for over six months were selected. They were clinically examined and blood from normal healthy soldiers was taken. Some workers at the Nutrition Research Laboratories were examined and have been included in the investigation. To date, data for 81 men are available, the averages and maximum and minimum figures are given in Table III :—

Table III.

Hæmatological examination of 81 healthy adult males in Nilgiris.

	Minimum.	Maximum.	Average.
(a) Age, years	18	43	24·3
(b) Period of residence at Coonoor or Wellington	3 months.	38 years.	...
(c) Weight, lb.	102	146	121.6
(d) Hæmoglobin g. per cent ...	13·1	20·3	15·87
(e) Red blood cells, mill /c.mm. ...	3·95	6·04	5·330
(f) Packed cell volume c.c. ...	38·0	65·0	49·03
(g) M.c.v., cu.μ	71·9	120·3	92·49
(h) M.c.h., γγ	23·5	40·8	30·00
(i) M.c.h.c., per cent	27·84	48·33	32·55
(j) White blood cells /c.mm. ...	3,200	10,800	7,271

(b) *Soya-bean milk feeding scheme.*—A repetition of the trial completed in December 1947 is under way. The experiments began in June 1948 and will conclude in December 1948. The clinical assessment of the response of the children to soya-bean milk as compared to cow milk is being done by the officers of the Nutrition Research Labortories; the actual feeding experiment is being conducted by Prof. V. Subrahmanyan and Dr. De of the Indian Institute of Science, Bangalore. The detailed report of the investigation will be submitted separately by the latter workers.

(c) *The incidence of stone in the urinary tract in India.*—With a view to ascertaining whether the changes in dietary habits resulting from the acute food shortage during the war years and after had any effect on the incidence of urinary calculi as ascertained in 1928, a questionnaire was circulated to the major hospitals in different parts of India through the courtesy of the heads of medical departments in various Provinces and States. The response on the part of the hospitals has been very poor. Out of 150 forms issued, only 41 were returned and even in these, information of dietary history was extremely meagre. Sind, North-West Frontier Province and West Punjab which were predominant stone areas as shown by the 1928 survey have now been separated from India and not much information has become available from them. Such returns as have been obtained in 1947-48 have been compared with returns from the same areas made in 1928. Although no conclusion of importance can be drawn from them, the following figures will be found interesting (Table IV) :—

TABLE IV.

Stones removed at operation.	Triennium.	
	1,926–28	1,944–46
Bladder	1871	1543
Kidney	44	82

On the basis of a closer scrutiny of the figures supplied by different areas, it does not appear as if the incidence of the disease has materially altered since it was first ascertained about twenty years ago.

IX. NUTRITION CLASSES.

In 1948, only one course was held from 15th March to 30th June. This was attended by medical graduates deputed by Provincial Governments and science graduates intending to specialize in nutrition research.

X. EDUCATIONAL AND ADVISORY WORK.

Numerous requests for information and advice were received throughout the year from all over India. Some of these involved analytical work which was only undertaken when information was needed by governmental agencies or other public organizations.

The Nutrition Museum is being re-organized and enlarged.

2. Enquiry on the formation, function and variations of plasma-proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

The clinical importance of plasma-proteins is due to the fact that they can be clinically measured. There are, however, pitfalls in the clinical interpretation of the values of plasma-proteins. Conditions associated with increased plasma volume will tend to give a lower value for different protein fractions of the blood plasma, even though the circulating protein is normal. On the other hand, dehydration tends to give a higher value despite the fact that the circulating proteins are depleted. For this reason hæmatocrit values and blood volume studies are necessary along with plasma-proteins.

A preliminary study of blood volume and circulating plasma-proteins in 29 cases of œdema.—These cases were clinically classified into 3 groups: (a) 15 patients with cardiac failure, (b) 8 cases of anæmia and (c) 6 cases of cirrhosis of liver. Various dyes have been used for the determination of blood volume, the most commonly used being Evans blue, T1824. As this was not available, congo red was used in this study.

Summary of results.

Group and number of cases	Total blood volume		Plasma volume		Total protein	Total protein	Albumin	Albumin	Globulin	Globulin
	Mean	c. c. / kg.	Mean gr.	c. c. / kg.	gr.	kg.		kg.		kg.
Normal (6)	4,557	82·9	2,644	48	198	3·6	125	2·27	72	1·30
Cardiac (15)	5,016	99·8	2,788	55	170	3·3	90	1·75	80	1·55
Anæmia (8)	3,055	82·3	2,231	60	116	3·1	57	1·54	58	1·58
Cirrhosis (6)	4,410	92·1	2,768	57	173	3·6	75	1·57	97	2·0

Cardiac cases.—In 13 of the 15 cases, there was an increase in the total blood volume, due to an increase in the plasma volume and cell volume. Eleven patients showed an inrcease of blood volume above 500 c.c. over expected blood volume calculated from body-weight. Five showed increase above 1,000 c.c. One patient with an increase of 1,247 c.c. died of pulmonary œdema. Five of the cases which were followed up showed a reduction in plasma volume with clinical improvement.

The lowered value of plasma-proteins observed in these cases is not due to dilution. There was an actual reduction in the circulating protein, particularly the albumin fraction, the globulin being slightly high. The plasma-protein values in cardiac cases were above the critical level at which œdema is likely to occur. In 2 patients the albumin was below 2 per cent, due possibly to associated malnutrition.

Anæmia cases.—In this group 7 of the 8 cases showed an increase in plasma volume varying from 100 to 1,285 c.c. and also a decrease in the cell volume. In spite of the increase in plasma volume, the blood volume was decreased in 5 cases. Walters *et al.* (1944) also reported a reduction in blood volume in 8 patients with anæmia. The mean body-weight in this group was only 37·1 kg.

The plasma-albumin was significantly below normal, the globulin remaining unchanged. In 3 cases albumin was below 3 g. per cent and in other 2, the albumin was below 3 g. per cent. In spite of the dilution due to increased plasma volume the trend of the values remains the same.

Cirrhosis group.—In this group the results were not uniform. Four of the 6 cases showed an increase in the plasma volume and also in blood volume. In 2 cases there was a decrease in the plasma and blood volume. The albumin was significantly low, the globulin was above normal in all cases. The trend of the results of plasma-proteins remains the same even when the effect of dilution due to an increase in plasma volume is taken into consideration.

3. Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

I. Fat metabolism.

(a) *Absorption of fats from intestines.*

It was stated in the last year's report that the absorption of fat from the human intestine seemed to be inhibited by the presence of monosaccharides. The presumption was that the phosphorylation of fat may be delayed by the presence of a monosaccharide which also requires a phosphate radical for its passage through the epithelial cells. The recent 'partition hypothesis' of fat absorption by Frazer (1946) and co-workers has given a serious blow to the old 'lipolytic' theory. The conception that phosphorylation is a stage in the process of fat absorption is open to question. According to the Frazer's partition hypothesis, neutral fat is absorbed as a very fine emulsion in the form of bile-salts-monoglyceride fatty acids complex. It has been shown that phospholipid is formed in the intestinal cells during fat absorption (Schmidt-Nielsen, 1946). The available evidence shows that the phospholipid in the intestinal cell should be more properly regarded as an end product than an intermediate compound in re-synthesis. More adequate information is, therefore, necessary as to the factors governing the phosphorylation. Experiments were, therefore, undertaken to see the effect of inorganic phosphate on the rate of absorption of fat from human intestine in the presence of a monosaccharide by using Frazer's chylomicron technique on blood serum. In some cases phosphate was found to increase the rate of absorption of butter which was delayed by the presence of a monosaccharide. Some elucidating figures are given in Table I :—

Table I.

Chylomicron count showing the Effect of an inorganic phosphate on the absorption of Fat from human intestine in the presence of a monosaccharide.

Times, hours.	1 oz. butter.	1 oz. butter+ 1 oz. glucose.	1 oz. butter+1 oz. glucose+2 g. Na_2HPO_4
0	7	6	6
1	24	11	12
2	19	15	20
3	18	41	18
4	7	10	10
5	2	10	8

The experiments must be repeated before one can arrive at a definite conclusion. The work is in progress.

It is known that vitamin deficiencies cause a decrease in the rate of absorption of fat due to general impairment of nutrition. Experimental evidence goes to point that in this respect certain vitamins exert more pronounced effect. It has been shown that when a fat is fed simultaneously with

5 gm. of choline hydrochloride (in water) the accumulation of fat in the intestinal cells which otherwise takes place, is less marked. Effcet of vitamins of B group is under investigation. Moreover, the difference in the absorption rates of various fats and oils might be due to the presence of lower fatty acids and essential fatty acids in the glyceride molecule. It is, therefore, proposed to see also the effect of these on the absorption of fats. Since the chylomicron technique has been successfully applied to albino rats, the latter experiments are being conducted on these animals.

(b) *Comparative nutritive values of vanacpati vegetable oil and butter.*

In continuation of the work reported last year on nutritive values of Dalda, ground-nut oil and butter (Polson), the effect of the basal diets was seen on the subsequent generations of albino rats as regards growth, reproduction, composition of body fats and composition of bone. Clinical symptoms, if any, were also recorded.

Growth.—Table II shows the increase in weights on the experimental diets of male rats.

TABLE II.

	Diet.	Average increase in weight.
16 per cent fat (9 per cent on wt. basis)	(a) Vanaspati (Dalda) ...	72
	(b) Grount-nut oil ...	47
	(c) Butter	61
25 per cent fat (23 per cent on wt. basis)	(a) Vanaspati (Dalda) ...	93
	(b) Ground-nut oil ...	46
	(c) Butter	63

The results so far obtained have shown that there was a slightly superior rate of growth in the animals fed on Dalda than on butter and ground-nut oil. This superiority was in both the groups, showing that Vanaspati is in no way inferior to other fats fed as regards the growth-promoting property in rats. It was also seen that the litters of both the groups (16 and 25 per cent fat) exhibited extremely poor rate of growth up to about 8 weeks.

Reproduction.—The reproductive capacity fell in the order of ground-nut oil, Dalda and butter. The ground-nut oil groups receiving 16 per cent as well on 25 per cent fat were found superior, the former being better than the latter. The Vanaspati and butter groups of the high fat diet showed extremely poor reproduction.

The growth curves of the females of the high fat diet (butter and Dalda group) showed one interesting feature. In some cases it was found that the rise in weight after mating was very slow and a subsequent fall in weight was not followed by the birth of a litter, probably showing the resorption of the fœtus. This point is under investigation by studying the histology of the sections of the reproductive organs in such animals.

Clinical symptoms.—Extensive alopecia on the neck and back was seen in animals receiving ground-nut oil and butter which was less marked in Dalda group. There were also cases of hæmorrhages from eyes and noses in all the groups receiving 16 per cent fat which evidently succumbed by developing spastic paralysis.

Composition of the body fat.—After obtaining the litters the parent rats were killed and the blood and the tissues of different organs (liver, brain, kidney, spleen and muscles) were analysed for total fatty acids, cholesterol and phospholipid. The iodine number of these fats and the calcium and phosphorus contents of the bones were also determined. Table III gives the analytical data regarding the distribution of the fat in different tissues in the rats kept on the diet for a period varying from 8 to 9 months :—

Table III.

Results : 15 per cent fat : Dalda and Butter—Parents.

Tissue		Cholesterol, mg. per cent		Total fatty acids, mg. per cent		I₂ No. (L.F. acids)		Total lipids, mg.		Phospholipids mg. per cent	
		Dalda	Butter	Dalda	Butter	Dalda	Butter	Dalda	Butter	Dalda	Butter
Blood	F	154	44	415	1,218	112	37	569	1,262	397	182
	M	108	41	519	2,444	104	79	619	2,488	187	312
Liver	F	..	112	..	3,618	..	76	..	3,730	..	1,757
	M	103	112	799	3,270	229	26	902	3,382	887	1,219
Muscle	F	88	74	2,894	4,674	103	57	2,894	4,748	430	314
	M	99	44	1,095	3,769	221	55	1,195	3,823	490	608
Brain	F	819	830	3,137	9,094	6	42	3,956	10,799	7,885	2,256
	M	773	642	3,272	4,744	13	97	4,045	5,386	2,515	1,095
Kidney	F	355	319	4,894	7,708	55	73	4,849	8,018	2,337	1,719
	M	227	275	2,102	3,765	117	97	2,329	4,040	1,300	1,923
Spleen	F	774	319	4,802	3,875	81	42	5,376	4,094	1,773	1,116
	M	126	206	734	2,937	12	12	860	3,143	1,603	1,077

It can be seen from the above results that the cholesterol contents of the tissues of rats fed with Dalda were slightly higher than those fed with butter, while in the case of total fatty acids it is just the reverse. As regards phospholipids, the two groups did not differ much considering the iodine number of butter fat and hydrogenated fat to be 38 to 60 (approx.) one can see that the body fat undergoes a process of saturation and de-saturation. This is more marked in the case of the Dalda group. The results seem to be in accordance with the findings of Barbous and others who believe that there is a threshold for saturated acids in the body fat (A. D. Barbous, *Jour. Biol.*, 1934 ; **16** ; p. 281). The work is in progress on the succeeding generations on Dalda and ground-nut oil.

As per suggestion from the N.A.C., this work was co-ordinated with that carried by the Vanaspati Research Planning Committee of the Food Ministry of the Government of India. The Officer in-charge attended the meeting of the Committee in May 1948 and presented the results of the investigation at the unit. The Committee took due notice of the findings and included the unit as one of the collaborating laboratories in their scheme for future work. Further experiments are in progress as per Committee's plan.

(c) *Nutritive values of pure-bred strains of cereals and pulses.*

As has been reported last year the study on the nutritive value of pure-bred strains of cereals and pulses grown in the presidency was continued. The estimations of the proximate principles, ash, calcium, phosphorus and iron together with the nicotinic acid and carotene were completed and the results have been published. Further work is undertaken for the estimations of

essential amino acids thiamine and riboflavin in the grains. It is contemplated to extend such studies on to other foodstuffs grown and consumed in the Presidency.

(d) *Nutritive value of milk and curds.*

The work on this problem was further continued and experiments were carried out to find out the relationship between the acidity, lactose and soluble and insoluble protein content of milk during curdling for varying lengths of time by mixed cultures. The mean results are shown in Table IV :—

TABLE IV.

Hours.	Acidity.	Lactose.	Soluble protein	Insoluble protein.
0	0·66	4·04	1·00	3·43
24	1·83	1·62	0·25	3·30
48	1·94	0·122	0·24	3·47
72	2·32	0·036	0·25	3·29

The results indicated that the acidity increased while there was a decrease in the lactose and soluble protein content. A few more experiments were performed to see whether the increase in acidity was proportional to the utilization of lactose by adding extra lactose. The results did not show any correlation between the two. Work is also in progress to note the changes in vitamins of the B group during curdling of milk by mixed and pure cultures.

(e) '*Physiological availability*' *of nutrients* (*studies in nicotinic acid*) *metabolism.*

Our knowledge regarding the different chemical reactions involved during the process of digestion and utilization of various nutrients is yet incomplete. This lack of knowledge is one of the main difficulties in the proper assessment of the nutritional potency of a foodstuff. A mere quantitative estimation chemically or physico-chemically of a nutrient would probably lead one to erronous conception about the nutritional potency of the foodstuff. Thus came to be introduced for the first time in the science of nutrition the idea of the ' physiological availability ' of nutrients. The idea has been rightly stressed by Elvehjem (1947) who pointed out that the difference between total content and available content of a nutrient must be understood.

Studies were undertaken to estimate the ' physiological availability ' of nicotinic acid from some vegetable foodstuffs. The preliminary experiments were reported last year and it had been shown that the liberation of nicotinic acid by enzymic hydrolysis from foodstuff gave lower figures than those obtained by the usual chemical method. The work was further continued with a view to assess the physiological availability of this nutrient for an organism. Experiments were carried out on albino rats. These were of three types : (1) Hydrolysis of foodstuffs by enzymic preparations from the gastric and intestinal mucosa of the rat, (2) Excretion of nicotinic acid in the animals on controlled diets and (3) *In vivo* digestion of foodstuffs in the animals. The results have shown that the nicotinic acid as estimated by chemical means was not completely available for nutrition. The excretion experiments have given a qualitative indication of this finding, while the *in vivo* digestion has supported the findings quantitatively. In Table V is shown the correlation between the chemical and *in vivo* digestion experiments.

TABLE V.

Foodstuff	Nicotinic acid content in µg per g.		Per cent availability.
	Acid hydrolysis.	*In vivo* digestion.	
Rice ...	56·5	34·0	60·1
Wheat ...	72·4	58·7	81·1
Gram ...	57·3	43·9	76·6
Tur ...	52·2	36·6	70·1
Yeast	419·2	387·2	92·4

Incidentally a method has been developed by *in vitro* enzymic hydrolysis which gave figures for nicotinic acid comparable with the *in vivo* digestion. Some results are shown in Table VI :—

TABLE VI.

Foodstuff (a)	Nicotinic acid content in µg. per g.		Diff. (b—c) (d)	Standard error of difference between the means (e)	Ratio (d : e) (f)
	in vitro enzymic hydrolysis (b)	*In vitro* digestion (c)			
Rice ...	27·7 ± 2·51	34·0 ± 3·25	±6·3	±4·1	1·5
Wheat ...	29·8 ± 1·48	58·7 ± 3·96	±28·9	±4·0	7·2
Gram ...	52·5 ± 3·14	43·9 ± 2·22	±8·6	±4·0	2·1
Tur ...	35·9 ± 2·23	36·6 ± 1·67	±0·7	±2·8	0·3
Yeast ...	263·8 ± 2·54	387·2 ± 4·64	±123·4	±5·3	23·8

Only in the case of yeast there is discrepancy between the figures. This question is under investigation. It is further intended to extend the work to other essential nutrients. Results of this investigation are being sent for publication.

(f) *The estimation of so called 'precursor of nicotinic acid' from vegetable foodstuffs.*

Elvehjem *et al.* (1944) have predicted the occurrence of nicotinic acid precursor in vegetable foodstuffs. The method of estimation of the substance is by absorption on charcoal and microbiological assay. These techniques are often time-consuming and difficult. A simple chemical method, therefore, was to be evolved. Attempts were made to this end and as per last year's report foodstuffs were incubated with acids, alkalis or buffers keeping in mind the properties of the precursor described by Elvehjem *et al.*

The results have shown that after incubation with the buffer of pH 1 for 3 hours at 37·5°C. there was almost complete extraction of nicotinic acid without the precursor. Thereafter the precursor underwent slow extraction and hydrolysis. There was simultaneous destruction of the nicotinic acid and the precursor so extracted after 6 hours. Similar was the case with the buffer of pH 10. In this case the hydrolysis of the precursor started very rapidly and a maximum value was reached after 6 hours giving a complete extraction

of nicotinic acid and the precursor. On the basis of these findings a tentative method has been evolved for the estimation of the nicotinic acid precursor from foodstuffs. The results by this method and by the absorption technique agreed fairly well. Table VII shows the amount of precursor in some foodstuffs by this method :—

TABLE VII.

Distribution of precursor in foodstuff materials.

Sample.	Nicotinic acid in μg per cent.		Precursor.	
	pH 1 and 3 hours.	pH 10 and 6 hours.	In μg. per cent.	Per cent of nictoinic acid.
Wheat ...	3,637·0	4,975·0	1,338·0	36·8
Wheat-bran ...	14,400·0	18,000·0	3,600·0	25·0
Rice ...	2,762·5	4,697·5	1,935·0	70·9
Jowar ...	882·5	1,842·5	960·0	108·8
Green gram ...	1,328·0	2,330·0	1,002·0	75·5
Pea ...	1,475·0	2,289·5	812·5	55·4
Gram ...	1,940·0	2,042·5	102·5	5·3

The results so far obtained are being sent for publication. Further work on other foodstuffs is in progress.

(g) *Researches on vitamin D.*

The phosphatase (alkaline) content of blood in rachitic rats was found to decrease even in rickets. The finding has been reported earlier from this unit. The observation has thrown doubts on osseous origin of serum phosphatase in rickets. Investigations were carried out to throw more light on this aspect of the problem by conducting experiments with the bone and serum phosphatases in rats to note the following :—

(a) The effect of different pH on the enzymes.
(b) The effect of different substrates.
(c) The stimulating action of inorganic ions.
(d) The stability of bone enzymes in normal and rachitic conditions.

The results are as follows :—

(1) Dis-similar fluctuations in bone and serum phosphatases in rachitic and normal conditions indicate that in both the conditions the bone was not the main source of serum phosphatase.

(2) A presence of a new neutral phosphatase active at pH 7 in bone and serum was suggested.

(3) Effect of different substrates showed the difference in the distribution of types of phosphatases in bone and serum at all times.

(4) The rachitic bone phosphatase was destroyed much more rapidly than the normal bone phosphatase. The presence of an anti-enzyme in rickets has been suggested.

The work is in progress as regards the histological studies on phosphatase by Gomori's technique and its correlation with biochemical findings. The results of these investigations are being sent for publication.

The laboratory has obtained recently a new Barcroft-Warburg manometric apparatus and it is intended to continue the work on the mode of action of vitamin D by respiratory technique.

4. Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

A. Studies in the biosynthesis of ascorbic acid.

It appeared from our work as reported before that pyruvate or some product of its metabolism might be a precursor in the biosynthesis of ascorbic acid. Thiamine and riboflavin were shown to be required for the process. The problem has been further pursued this year on the following lines:—

(i) Attempts to accelerate the biosynthesis of ascorbic acid with hydroxypyruvic acid (which might be an intermediate in the production of ascorbic acid from pyruvic acid), thiamine and riboflavin being provided as catalysts for the process.

(ii) Study of the possibility of the *in vitro* synthesis of ascorbic acid from hydroxypyruvic acid in presence of dilute alkali.

(iii) Study of the effect of the ultra-violet irradiation on pyruvic or hydroxypyruvic acid in presence of thiamine, riboflavin, or a photosensitizer such as chlorophyll.

(iv) Study of the stimulating effect of sunlight on the formation of a reducing substance during germination of seeds and the biological assay of the product of irradiation to find whether it is identical with vitamin C.

(i) *Attempts to accelerate the biosynthesis with hydroxypyruvic acid.*—In course of a recent study of the deamination of dl-serine by rat kidney slices, Sprinson *et al* (*Jour. Biol. Chem.*, 1946, 164, p. 411) isolated a new 2 : 4-dinitro-phenylhydrozone which fulfils the analytical requirements of a derivative of hydroxypyruvic acyl. They then prepared this acid and found it to be very reactive, giving rise to a multiplicity of reaction products under certain conditions. They further suggested that the reactivity of the acid coupled with its occurrence in some biological systems as observed by them, may prove of much interest. In animals known to synthesize vitamin C, it seemed possible that the process involves certain reactions depending on hydroxypyruvic acid. It was, therefore, considered desirable to study the effect of hydroxypyruvic acid on the biosynthesis of vitamin C by the rat. For that purpose, a concentrate of H.P.A was prepared according to Sprinson *et al.* (*loc. cit.*) by hydrolysing a known amount of bromo-pyruvic acid with a known strength of NaOH. The preparation which was 0·05 M with respect to H.P.A was further concentrated to 0·3 M under reduced pressure in an atmosphere of nitrogen. Well-fed healthy rats (90 g. to 110 g.), liberally dosed with thiamine and riboflavin were given a daily supplement (2 c.c. to 3 c.c.) of H.P.A. (0·3 M) by mouth, for 7 to 10 days. Another group of animals was also injected daily with (0·5 c.c. to 1·0 c.c.) H.P.A for the same period. During this time, 24 hours' urine was collected from all groups of animals (rejecting the first two days collection) and tested for ascorbic acid by the usual chemical method. Both the urinary excretion of ascorbic acid and the reserve of vitamin C in liver and kidney tissues were unaffected by the administration of H.P.A.

(ii) *Attempts at the* in vitro *synthesis of vitamin C from hydroxypyruvic acid.*—It has been observed by Sprinson *et al.* (*loc.cit.*) that hydroxypyruvic acid kept in contact with weak alkali (0·01 N) undergoes a rapid and complex series of tautomeric changes and condensations. Two molecules of H.P.A ($C_3O_4H_4$) may conceivably give rise to one molecule of ascorbic ($C_6O_6H_8$). It was, therefore, considered interesting to see whether any ascorbic acid is formed in the reaction mixture, when hydroxypyruvic acid is exposed to dilute alkali (0·01 N) However, when hydroxypyruvic acid (0·3 M) was kept in contact with 0·01 N sodium hydroxide at room temperature or refluxed over water bath for varying periods (3 to 6 hours), no indophenol-reducing substance could be detected in the reaction products.

(iii) *The study of the effect of ultra-violet irradiation on pyruvic or hydroxy pyruvic acid in presence of thiamine and riboflavin or chlorophyll.*—Solutions of pyruvic acid or hydroxy pyruvic acid (0·3 M) were exposed to ultra-violet irradiation (a) alone, (b) in presence thiamine and riboflavin and (c) in presence of thiamine, riboflavin and chloryphyll for varying periods (3 to 6 hours), at a temperature of 28°C. to 30° C. At the end of the exposure the solutions were tested for vitamin C. No indophenol-reducing substance could, however, be detected in the irradiated solutions.

(iv) *Study of the effect of sunlight and ultra-violet light on the formation of ascorbic acid during germination of seeds and the biological assay of the product so formed.*—The formation of ascorbic acid during germination of seeds and the stimulating effect of light on the process have been reported by us before. It has been observed that an indophenol-reducing substance accumulated during germination in the dark and increased to a considerably higher value (20 to 25 per cent in case of *Phaseolus mungo*). When the seedlings are exposed for a period to direct sunlight or ultra-violet rays of wave lengths between 3,000° and 4,000° A. U. the indophenol-reducing substance so formed in *Phaseolus mungo* during germination and exposure to light has now been found to be identical with ascorbic acid by biological assay with guinea-pigs. The study of the effect of this irradiation on the germinating seeds has been extended to the other varieties of pulses such as *Cicer arietimen*, *Phaseolus radiatus*, *Lens esculenta*, etc. It has been observed that though there is formation of ascorbic acid during the germination of these pulses in the dark, the effect of irradiation (with both sunlight and ultra-violet light) is not very pronounced except in case *mas-kalai* (*Phaseolus radiatus*) where a slight increase is found.

B. Studies in the biosynthesis of fat with special reference to thiamine and riboflavin.

Both thiamine and riboflavin are known to participate in the biosynthesis of fat (McHenry, *Jour. Biol. Chem.*, 1938, **125**, p. 653). This effect can, therefore, be studied in animals rendered deficient with respect to these vitamins. It has been observed by us and others (Ochoa *et al.*, *Biochem. Jour.*, 1939, **33**, p. 2008) that even when liver is very nearly depleted of thiamine and riboflavin, the rat even in highly acute stage of deficiency retains in other organs, particularly the brain and kidney, a considerable amount of these substances (nearly normal values). The microflora present in the intestine of the rat probably make up the deficiency by synthesis and supplying the vitamins for storage in those organs. Sulpha drugs are known to destroy many of the intestinal microflora which synthesize thiamine and riboflavin. So, by administering

small amounts of a sulpha drug, particularly sulpha-guanidine, to the rat during thiamine and riboflavin deficiency, a more drastic depletion can be achieved and its effect on the biosynthesis of fat is likely to be more pronounced. Experiments are now in progress to find whether under this condition the formation of fat would be further inhibited.

It has also been observed by us that there is a partial depletion of vitamin C in the tissue reserve of rats when they are rendered thiamine or riboflavin deficient. It is possible that the observed diminution in fat synthesis in thiamine and riboflavin deficiency might be connected with the deficiency of vitamin C caused by the lack of thiamine and riboflavin. Studies are in progress to see whether this is the case.

C. Fatty acid oxidation by the animal.

Lang in a series of papers (*Ztschr. Physiol. Chem.*, 1939, **261**, p. 249 ; *ibid.*, 1939, **262**, pp. 121 and 123) described an enzyme obtained from rat liver by extraction with phosphate at pH 8·2. It acts as a dehydrogenase upon fatty acids with both odd and even numbers of carbon atoms, the activity increasing with the length of the carbon chain. The system has little effect on unsaturated fatty acids and appears to be specific for the saturated higher ones. The products of oxidation are shown to be unsaturated fatty acids. A co-enzyme has also been found to be necessary. Lehninger (*Jour. Biol. Chem.*, 1942, **143**, p. 147) has made attempts to obtain by extraction from kidney and muscle tissue an enzyme system free from cells and active in the oxidation of acetoacetate. Feeble but definite activity has been found in his preparations. It appears, therefore, that a more fundamental approach to the problem of the metabolism of fatty acids can be made from the standpoint of enzymes and other associated factors such as co-enzymes, inorganic ions, etc.

The present work was undertaken in order to explore the mechanism of the oxidation of liver fat with particular reference to the enzyme systems involved. The study of the dehydrogenase system present in the rat-liver tissue was made with the methylene blue technique of Thunberg. The enzyme preparation was made with the washed minced fresh liver tissue of the rat and studied with palmitic acid as a substrate, boiled muscle extract (b.m.e) being used as a source of the co-enzyme. The results are given in Table I :—

Table I.

The dehydrogenase activity of the normal washed minced rat-liver tissue towards palmitic acid as a substrate by the Thunberg technique. The time (mins.) for 80 per cent reduction of colour of 0·2 c.c. methylene blue is recorded.

Experiment number.	With substrate and b.m.e.*	Without substrate and with b.m.e.*	With substrate without b.m.e.*	Without substrate and without b.m.e.*
1.	40	65	200	200
2.	62	85	200	200
3.	52	75	200	200
4.	43	63	136	200
5.	37	43	200	200

* b. m. e.=boiled muscle extract.

The results in Table I indicate that there is a definite dehydrogenase present in the liver tissue of rats.

The study of the oxidation of fatty acids by the surviving liver tissue (normal or rendered abnormal by vitamin deficiency, endocrine disfunction, etc.) was then made by the manometric technique of Warburg. The results are given in Tables II and III :—

TABLE II.

The oxidation of fatty acids by normal guinea-pig liver slices by the Warburg manometer. Temp. 37·5°C.

Substrate.	Concentration.	Mean Q_{O_2} :	
		With substrate.	Without substrate.
Butyrate ...	0·16 M	5·0	2·9
Caprylate ...	0·096 M	8·4	5·7

The results agree with those obtained by Quastel *et al.* (*Biochem. Jour.*, 1935, **29**, p. 2143).

TABLE III.

Oxidation of caprylic acid by the liver tissue (slice) of the normal and scorbutic guinea-pigs by the Warburg manometer. Temp. 37·5°C.

Q_{O_2} normal liver :			Q_{O_2} scorbutic liver :		
With sodium caprylate.	Without sodium caprylate.	Per cent increase.	With sodium caprylate.	Without sodium caprylate.	Per cent increase.
7·50	5·7	31·0	7·02	4·91	44·0
6·02	3·75	60·0	9·80	6·93	41·0
12·26	7·82	56·7	5·74	4·12	39·0
8·10	5·50	47·3	7·90	4·95	59·0

Thus, the oxidation of caprylic acid by the liver tissue is not significantly affected by the scorbutic condition of the animals. In a preliminary experiment with castrated male rats the following points have been noted :—

(a) The amount of liver fat increases with a corresponding decrease in iodine number.

(b) The rate of oxidation of caprylic acid is gradually depressed falling to half the normal value after 4 weeks, observation made after 6 months showed that the oxidation has completely stopped.

D. STUDIES IN FOOD-IRON.

(1) *Biological availability of the iron of wheat.*—It was reported last year that iron of rice and lentil is poorly absorbed from the intestinal tract in comparison with ferric chloride. This year the biological availability of food-iron has been studied in wheat by the feeding and balance-sheet experiments with rats grouped as follows :—

GROUP I: Receiving wheat only.

GROUP II: Receiving wheat and a supplement of 0·03 mg. copper (as copper-sulphate solution) dissolved in 0·1 c.c. of water per rat per day. The purpose of the second group was to see the effect of copper on the absorption of iron of wheat from the intestinal tract.

Each group consisted of 9 rats, 4 males and 5 females, of weight between 75 g. and 83 g. Period of study extended over 10 days. Wheat with iron content 8·9 mg. per 100 g. dry weight was given *ad lib.*

The results indicate that :—

(i) Like iron of rice and lentil, iron of wheat is poorly absorbed in comparison with ferric chloride (absorption of iron of wheat being 20 to 25 per cent, the figure for that of ferric chloride fed at a level of 0·5 mg. per rat per day is 68 to 72 per cent).

(ii) Copper has no augmenting effect on the absorption of iron in wheat.

(2) *Haemopoietic effect of the iron of some materials : wheat, egg-yolk, rice and ferric phytate.*—It has been shown by Rose *et al.* (*Jour Nutr.*, 1936, **11**, p. 31) that wheat and other cereals are important dietary sources of iron. On the other hand, Elvehjem *et al.* (*Jour. Biol. Chem.*, 1933, **103**, p. 61) concluded that iron of wheat was half as effective as inorganic iron in haemopoiesis. As wheat is a major dietary ingredient in some parts of India, a thorough investigation on the haemopoietic effect of iron in wheat is of considerable importance.

The experiments of Sherman *et al.* (*Jour Biol. Chem.*, 1934, **107**, p. 289) have shown that iron of egg yolk is utilized to the same extent for haemoglobin regeneration as inorganic iron. By following the feeding and balance-sheet experiments on normal rats it was shown last year that iron of egg-yolk as such or of its lecithovitellin protein fraction is absorbed from the intestinal tract to the same extent as that of ferric chloride when fed at the same level with respect to iron. As haemoglobin regeneration assay provides an additional criterion for the biological evaluation of food iron, this was also undertaken with egg-yolk.

Though rice and lentil contain appreciable amounts of total iron (rice 6·1 mg. per cent, lentil 11·4 mg. per cent on dry basis) the biological value of this iron by hæmoglobin-regeneration method has not been studied. In fact, there is very little work on the evaluation of Indian foods so far as hæmoglobin regeneration is concerned, though data are available on the total iron contents and their ionizable fraction in different foodstuffs. Hæmopoietic activity of iron of rice was undertaken as rice forms the major dietary constituent for a large population in India.

It has been observed last year that cereal grains, such as wheat and rice, and pulses, such as lentil, contain considerable amounts of phytin phosphorus. In order to see how far this phytic acid interferes with the absorption of iron by the intestine, feeding and balance-sheet experiments were carried out before with ferric phytate. It appeared from our results that ferric phytate is poorly absorbed by the intestinal tract in comparison with ferric chloride, the level of iron fed being equal in both the cases. In the present experiments, biological availability of iron of ferric phytate was undertaken, the method of hæmoglobin regeneration in anæmic rats being followed.

Rats were made anæmic (hæmoglobin level 60 to 65 per cent) according to he technique of Elvehjem *et al.* (*Jour. Biol. Chem.*, 1931, **93**, p. 189). When he rats were 35 to 42 days old, the hæmoglobin level generally came down to 0 to 65 per cent. The rats were then divided into groups with respect to itter, sex and weight. Each group consisted of 6 rats, excepting the first one vhich consisted of 4 rats.

Diet of different groups (i) GROUP I : Each rat of this group was given cow's milk *ad lib.* ogether with a supplement of 0·8 mg. of iron as ferric chloride and 0·03 mg. copper as copper ulphate dissolved in 0·1 c.c. of acidified water.

(ii) GROUP II : Each rat of this group was given cow's milk *ad lib.* together with a upplement of 0·3 mg. iron as ferric phytate and 0·03 mg. copper as copper sulphate solution uspended in 2-c.c. portions of cow's milk. When the rats had taken the supplement, they ere given their quota of milk.

(iii) GROUP III : Each rat of group (iii) was given cooked rice diet containing 3 per cent Brewer's yeast and 3 per cent Steenbock's salt mixture No. 40 without iron (on dry basis) *ad lib.* and 3 c.c. of 10 per cent Klim powdered whole milk mineralized with respect to copper 0·03 mg. copper). The amount of diet taken by each rat varied between 3 and 7 grammes upplying 0·18 mg. to 0·42 mg. of iron per rat per day of which 8 per cent came from rice. Unless Klim and yeast were supplied, growth could not be maintained. The iron content of east and Klim milk powder are 4·0 mg. and 3·0 mg. per cent respectively.

(iv) GROUP IV : Each rat received whole wheat *ad lib.*, each rat taking 3 g. to 6 g. of vheat daily, which supplied 0·28 mg. to 0·5 mg. of iron. Copper supplement was given rally—dose being the same as with other groups.

(v) GROUP V : Each rat was given 2 g. to 3 g. of dried egg-yolk (iron content 13·1 mg. er cent) supplying 0·26 mg. to 0·39 mg. of iron and cow's milk *ad lib.* Cow's milk was miner-lized with respect to copper (0·03 mg. copper per 20 c.c. of milk).

All the rats were given two drops of Adexolin-Glaxo per rat per week. Period of experiment extended over 40 days. Hæmoglobin was measured by Newcomer's method once in 10 days with blood obtained from a cut at the tip of the tail.

The results indicate that the available iron (for the hæmoglobin regeneration) in egg-yolk is 94 per cent, the available iron in rice is 45 per cent, the available iron in wheat is 62 per cent and the available iron in ferric phytate is 52 per cent (all the figures have been calculated on the basis of ferric chloride being taken as 100).

(3) *Ionizable iron in rice, wheat and egg-yolk.*—The ionizable iron as measured by α-α' dipyridyl method was tested for its biological availability for hæmoglobin regeneration. The results with wheat are from 4 varieties and those of rice from seven varieties, six parboiled and one *atap*. The total iron content of wheat varied between 6·4 mg. and 9·3 mg. per cent of which the ionizable fraction was 3·1 mg. to 4·2 mg. The total iron contents of rice varied between 2·0 mg. and 6·1 mg. The ionizable fraction with parboiled rice varied between 0·1 mg. and 0·9 mg. per cent and with *atap* rice it was 0·8 mg. per cent. Egg-yolk (duck's) contained 12·8 mg. per cent ionizable iron, its total iron content being 13·1 mg. per cent.

(4) *Effect of rice on the storage of iron in the body, liver and spleen of rats.*—In order to verify whether poor hæmopoiesis with iron of rice results from poor absorption by the intestinal tract, work on the estimation of iron of body, liver and spleen of rats maintained on rice diet was undertaken. If the rats absorb iron of rice poorly in the intestinal tract, rats kept on rice diet will show lower iron content in liver, spleen and body in the long run. With this object 3 litters of rats were taken. At the age of 21 to 25 days, they were kept on an exclusive milk diet. When 49 to 53 days old, one rat from each litter was sacrificed and

the iron contents of liver and spleen were estimated, while the remaining rats were grouped into two groups: one of 7 rats receiving rice diet (94 g. rice, 3 g. yeast and 3 g. Steenbock's salt mixture 40, without iron and supplement of 0·03 mg. copper per rat per day given with 3 c.c. of milk separately, 98 per cent of iron of diet coming from rice), the other of 6 rats receiving milk *ad lib.* along with a supplement of 0·4 mg. to 0·6 mg. iron as ferric chloride and 0·03 mg. copper as copper sulphate per rat per day, dissolved in 0·1 c.c. of acidified water. Cod-liver oil (Adexolin-Glaxo) was given in a dose of 2 drops per rat per week. Rice diet taken by each rat varied between 7 g. and 10 g. dry weight and iron taken by each rat between 0·41 mg. and 0·59 mg. At the age of 84 to 88 days the rats were sacrificed and then the total iron in liver, spleen and body was estimated. Weekly weights and hæmoglobin records were also kept.

The results show lower value for the total iron contents of liver, spleen and body of rats maintained on rice diet and they corroborate our previous findings that iron of rice is but poorly absorbed in the intestinal tract. It appears that there is a slight fall in the hæmoglobin level with the group of rats maintained on rice diet, though the total iron content in the liver and spleen was not so low as to effect that. The failure may be due to lack of certain proteins in rice which help utilization of iron for hæmopoiesis.

(5) *Effect of iron free egg yolk (duck), albumin and casein on the hæmopoietic effect of iron of rice.*—It is well known that ferric iron forms complexes with phospho-protein of egg-yolk, casein and serum (Tompsett., *Biochem. Jour.*, 1934, 28, p. 1802). Iron of at least one such phospho-protein system (egg-yolk) is biologically very active as judged from our hæmopoietic study and balance-sheet experiments. It was, therefore, considered possible that the addition of different proteins in the rice diet might help in the utilization of the iron of rice. Three proteins were tried, viz. egg-yolk (duck), egg-albumin and casein. The results indicate that egg-yolk added to rice diet greatly augment the low hæmopoietic effect of iron of rice, while the effect of egg-albumin and casein is not very pronounced.

(6) *The effect of fat on the hæmopoietic activity of iron of rice.*—In order to find whether fat has any augmenting effect on the hæmopoietic activity of iron of rice, a group of anæmic rats (six in number) were maintained on the diet of the following composition: Rice 59 g., oil (butter-fat, Polson) 35 g., yeast 3 g. and salt mixture 3 g., containing 5 mg. of iron per 100 g. dry diet. It has been observed that high fat tends rather to depress the hæmopoietic effect of iron of rice diet which itself is very low. The difference, however, may not be significant.

5. Enquiry on the evaluation of proteins in terms of hæmopoietic activity and the relation of dietary protein to blood and muscle hæmoglobin under Professor M. Damodaran at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

Experiments were planned to study the hæmoglobin and r.b.c. formation in animals maintained at different levels of protein intake for more than one generation. The low protein diet was based upon rice flour and contained 5 per cent of rice protein as the sole source of nitrogen. As animals on this diet failed to produce young, some attention was also given to the relation of dietary protein to reproductive functions.

In the first completed set of experiments which lasted seven months, two groups of rats were used with 12 animals in each group. Group I received

a diet containing enough rice flour to supply 5 per cent of protein. The diet for Group II contained 3 per cent casein in addition to the rice protein. The composition of the two food mixtures is given below :—

	Group I. Per cent.	Group II. Per cent.
Rice flour (polished rice) ...	70	70
Sugar	16	13
Fat	10	10
Steenbock salt mixture ...	4	4
Casein		3

Estimation of hæmoglobin and r.b.c. were made every fortnight in the earlier stages of the experiment and at longer intervals later. Weekly records of food intake and weight were maintained. After the animals were placed upon the experimental diet, the stage of the ostrus cycle was determined by daily microscopic examination of the vaginal smear. Successful mating was ascertained by examination for plug and semen in the vagina.

TABLE I.

		Initial.	3 months.	7 months.
5 per cent protein :				
Group I	R.b.c. ...	6·71± 0·34	6·63± 0·27	6·28± 0·14
	Hb ...	14·46± 0·46	13·18± 0·28	12·35± 0·90
	Weight ...	106·5 ± 15·49	119·0 ±14·2	111·5 ±31·26
8 per cent protein :				
Group II	R.b.c. ...	6·73± 0·29	7·32± 0·46	7·31± 0·47
	Hb ...	14·50± 0·54	14·69± 0·86	14·50± 0·96
	Weight ...	110·5 ± 17·88	190·0 ±15·0	146·7 ±14·23

From the summarized results in Table I it will be seen that the Group I animals on low protein diet showed distinctly lower r.b.c. and Hb value in comparison both with initial values as well as of the animals in Group II receiving the 3 per cent casein supplement. On the low protein diet weight gains were irregular and often negligible ; the animals in the second group showed a much steadier growth but even in these optimal weight increases were not obtained.

The most striking effect of the low protein diet was the failure to breed. In all except one animal out of the 8 females in Group I mating was unsuccessful; the one exception gave birth to a litter of 5 all of which died within an hour after birth.

These observations are in keeping with previous findings on the influence of protein on reproduction (Evans and Bishop, 1922 ; Courrier and Raynaud, 1934 ; Guilbert and Goss, 1932).

The results obtained have been confirmed in a second set of experiments which have now been in progress for 3 months.

TABLE II.

Group number.	Protein, per cent.		Initial.	84 days.
I	5	R.b.c. ...	6·51 ± 0·32	6·72 ± 0·25
		Hb ...	14·65 ± 0·25	13·32 ± 0·33
		Weight ...	101·1 ± 11·84	122·4 ± 14·03
II	8	R.b.c. ...	6·58 ± 0·25	6·90 ± 0·16
		Hb ...	14·13 ± 0·15	14·37 ± 0·37
		Weight ...	102·0 ± 17·7	182·1 ± 10·0
III	30	R.b.c. ...	6·48 ± 0·29	7·09 ± 0·13
		Hb ...	14·26 ± 0·33	14·70 ± 0·38
		Weight ...	106·2 ± 6·27	150·3 ± 12·28

In this experiment an additional group of six animals was maintained on a 30 per cent protein diet; but so far they have derived no marked beneficial effect with regard to r.b.c. and haemoglobin, from the higher level of protein.

6. Enquiry entitled 'Preparation of anti-rachitic substances' under Professor B. V. Bhide at the Maharaja Pratap Sinh, Chemical Laboratory, S. P. College, Poona.

I. In continuation of the last year's work, 4 acetoxy-2-cinnamylidene-cyclohexanone (m.p. 170°C.) has been prepared in larger quantities. It was then subjected to Reformatsky's reaction with ethyl bromoacetate. The resulting crude hydroxy ester was directly hydrolysed to 4-hydroxy-2-cinnamylidene-cyclohexan-1-ol acetic acid (m.p. 180°C. to 182°C.). This acid on boiling with acetic anhydride gave a de-hydrated acid, 4-acetoxy-2-cinnamylidene-cyclohexylidene acetic acid (m.p. 189°C. to 190°C.), which was de-carboxylated by heating under reduced pressure when a neutral product was obtained. It was directly hydrolysed when a dark-brown solid was obtained which melted indefinitely between 60°C. and 75°C. and was in such poor yields that it could not be further purified nor could it be crystallized. It is, however, believed to be methylene-2-cinnamylidene-4-hydroxycyclohexane.

Biological assay.—

(1) 1-methylene-2-cinnamylidene-cyclohexane.
(2) 1-methylene-2-citrylidene-cyclohexane.
(3) 1-methylene-2-cinnamylidene-4-hydroxy-cyclohexane.

The above three compounds were tested by Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor and found to possess no anti-rachitic properties.

II. The work described here is concerned with the synthesis of compounds possessing structure similar to rings C and D in calciferol.

4-hydroxy-hydrindone will be an interesting intermediate in the synthesis of 1-keto-4-hydroxyhexahydro-indane. Attempts to prepare it by the cyclization of 2-methoxy-6-carboxyl-phenyl propionic acid have failed. Finally it has been synthesized from 4-amino-hydrindone through Sandmeyer's reaction. It was obtained as fine needles crystallizing from alcohol (m.p. 235°C.). It has been characterized through its semi-carbazone (m.p. 205°C.)

Attempts were also made to prepare 8-methyl-4-methoxy-1-keto-hexahydro-indane by the cyclization of β (2-methyl-6-methoxy-1-cyclohexanyl) propionic acid, but all of them have failed so far.

III. It was necessary to prepare calciferol phosphate with a view to study the mechanism of calcification. Preliminary experiments were, therefore, made to prepare cholesterol phosphate. It has been prepared by direct esterification with ortho phosphoric acid. It is a hygroscopic solid which melts at 183°C. to 185°C. It seems to be different from the one (m.p. 175°C.) reported by Wagner-Jauregg, Lennartz and Kuthny (*Ber.* 1941, **74B**, pp. 1513-21) for which they prefer di-cholesteryl-ortho-pyro-phosphate structure. The solid (183°C. to 185°C.) is in all probability cholesterol orthophosphate. The analysis for phosphorus agrees with the proposed structure. Attempts are being made on similar lines to prepare calciferol phosphate.

IV. To see whether the triene system in calciferol is essential for antirachitic activity, octahydrocalciferol has been prepared.

7. Bangalore school feeding scheme and enquiry on soya-bean milk under Professor V. Subrahmanyan and Dr. S. S. De at the Indian Institute of Science, Bangalore.

Since the report was last submitted, further work has been done in continuation of the earlier experiments to study the nutritive value of soya-bean milk and curd in comparison with cow's milk and curd. The earlier experiments that had been started in the orphanages and convents were completed in last December and the results were statistically analysed. After the results were thus analysed, it was suggested that the same experiments must be continued for another six months using younger subjects. Accordingly, experiments were started in June 1948 on 200 subjects in the age group 6-10 years in four convents. These experiments have been in progress for the last four months and would be completed by December 1948.

The feeding experiments with soya-bean curd that were being conducted in the schools in the Civil Station, Bangalore, had to be stopped in March 1948 since the schools were closed for the summer vacation. These experiments could not be resumed in June after the schools re-opened since there was considerable delay in the provision of grants for the continuation of the feeding scheme by the Mysore Government. It was, however, felt that actual experimental data had to be collected before the value of the school feeding scheme with soya-bean curd could be demonstrated. Hence fresh experiments were started in the Government Boys' Middle School, Malleswaram, Bangalore, which is near the Indian Institute of Science. The experiments have been in progress for the last two months and will be continued till December.

Metabolism experiments on human subjects were conducted to study the utilization of calcium in soya-bean curd fortified with calcium hydrogen phosphate.

The value of soya-bean milk as a food for infants has also been studied. Actual metabolic data on the utilization of protein, calcium and phosphorus have been collected on six children.

A method of preparing milk from soya-bean powder has been standardized.

The various experiments that have been started and those that have been completed are outlined below. The results of the experiments are also given :—

1. *Human feeding experiments with soya-bean milk.*—Two hundred subjects below 9 years of age have been selected in four convents and receive soya milk or cow's milk as a supplement. The plan of the experiment conducted this year includes three groups :—

Group A (80 children)	...	12 oz. cow's milk as supplement.
Group B (80 children)	...	12 oz. soya milk as supplement.
Group C (40 children)	...	Control—(no supplement).

These experiments were started in last June in consultation with Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor, and the Statistician, All-India Institute of Hygiene & Public Health, Calcutta.

In addition to recording of heights and weights, systematic record of nutrition deficiency is being maintained on clinical score card. The r.b.c. and hæmoglobin levels of the subjects are being determined at periodic intervals. The results of observations made by the Medical Officer till now will be presented by the Director, Nutrition Research Laboratories, Coonoor. The final analysis of the results will be conducted by the Statistician at the completion of the experiments in December next.

2. *Feeding experiments with soya-bean curd and rice in Government Boys' Middle School, Malleswaram, Bangalore.*—These experiments were started on 300 subjects in the age group 6-12 years in August in a local school. The subjects were grouped statistically into three groups of 100 each. The plan of the experiment includes :—

Group A (100 subjects)	...	8 oz. cooked rice+8 oz. soya-bean curd.
Group B (100 subjects)	...	8 oz. cooked rice+8 oz. skimmed milk curd.
Group C (100 subjects)	...	Control—(no supplement).

Increases in height and weight are being noted at monthly intervals. Regular clinical observations are being made by a Medical Officer. The results obtained till the end of December 1948 will be statistically analysed and treatments A and B will be compared against treatment C and also between themselves.

3. *Infant feeding experiments with soya-bean milk.*—About 30 children ranging in age from six months to three years were selected in a residential orphanage and they were fed soya-bean milk for three months. The infants below one year were given soya milk as the sole food. The others received one pound of soya-bean milk along with their usual food which consisted of boiled rice and vegetable curry and soup.

The following general observations which were made in collaboration with the Medical Officer to the orphanage will be of interest :—

(a) The infants and the toddlers had no difficulty in digesting the milk. No digestive disorder was manifested. Even those subjects who had digestive troubles in the beginning of the experiment responded remarkably well to soya-bean milk feeding.

(b) The general health of the subjects was improved. All the subjects put on weight.

Apart from these general observations, metabolic data on a representative group of six subjects were collected. These data relate to the balances of nitrogen, calcium and phosphorus during two metabolic periods. In the first period the children received soya-bean milk as their sole food. During a subsequent period, they received cow's milk as their sole food. From the data on the intakes and excretions during a one-week period, the percentage retention of nitrogen, calcium, phosphorus was calculated for the two milks. The final results are tabulated below :—

Average percentage retention of nitrogen, calcium and phosphorus.

	Nitrogen.	Calcium.	Phosphorus.
Soya milk ...	60·7	52·4	46·6
Cow's milk ...	70·3	51·8	49·2

On account of the inherent difficulties in carrying out metabolism experiments on infants, further data on the children could not be collected.

4. *Utilization of calcium from soya-bean curd in human subjects.*—These experiments were conducted on six adult human subjects. The utilization of calcium in soya-bean curd fortified with calcium hydrogen-phosphate was compared with that of calcium in cow's milk curd. The outline of the experiment was to compare the calcium balance of the subjects during three metabolic periods of ten days' duration each. During the first period a low calcium diet was served to the subjects. Cow's curd and soya-bean curd were given as supplements to the low calcium diet during periods two and three respectively. From a study of the calcium intakes and balances during the three periods the percentage utilization of calcium from cow's curd and soya curd was calculated. The average of the results for six subjects is tabulated below :—

(The figures represent daily averages in mg.)

Curd supplement given.	Calcium intake.		Calcium excretion.			Percentage utilization of the calcium of curd.
	Basal.	Curd.	Urine.	Fæces.	Total.	
...	327	...	105	237	342	...
Cow ...	327	508	145	585	730	27·0
Soya ...	327	510	145	597	742	24·9

5. *Experiments with soya-bean milk at the Sri Krishnarajendra Hospital, Mysore.*—On account of its low percentage of fat, and the ease with which it can be digested, soya-bean milk has been recommended for patients suffering from the various affections of the digestive tract. Childern suffering from hepatic cirrhosis are being used as subjects in Mysore and the suitability of soya milk as a food for such subjects is being studied. Since soya-bean milk contains a low percentage of fat and since the cirrhotic children suffering from fatty degeneration of the liver require a diet as much low in fat as possible, soya milk is being used as food. A similar group of subjects is being treated with cow's milk. The experiments have been started about a month ago. The recovery of the subjects from the disease is being watched. It is also proposed to carry out balance studies with respect to fat and protein on the children.

The two main field experiments that are being conducted will be completed in December. The results of both these experiments will then be statistically analysed and the conclusions drawn from the data will be embodied in a separate report.

8. Enquiry on plant carotenoids in relation to their biological importance under Professor M. Damodaran at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

During the current year, work has been carried out on the following problems :—

(i) *Provitamin A activities of neo-β-carotene B and neo-β-carotene U.*—Neo-β-carotene B was found to be 45·2 per cent as active as its all-trans isomer, while neo-β-carotene U exhibited a potency which corresponded with 18·5 per cent of that of all trans β-carotene.

(ii) *Changes in the carotenoid pigments during development.*—The development of carotenoid pigments during ripening were studied in the case of mangoes, tomatoes and leafy vegetables. In the case of mangoes, three different types were studied namely Saroli, Cheunsa and Delhi Desi variety mangoes. The results of chromatographic separation and estimation of the carotenoid pigments had shown that all the carotenoids increase during ripening. In Saroli mangoes, β-carotene is synthesised at a greater rate than any other pigment, while both in Cheunsa and Delhi Desi variety mangoes, the increase in the amount of xanthophyll and β-carotene is almost of the same order.

Changes in the carotenoid pigments during the ripening of tomatoes under varying environmental conditions were determined with a view to find out the best means of increasing the β-carotene content. Vine-ripened fruits were found to be richer in carotene than fruits of the same variety picked green and ripened during storage. Lycopene formation at temperatures $< 30°$ C. was not affected by subjecting the tomatoes for short period to higher temperatures up to 35° C. The β-carotene content of tomatoes was but little influenced by the environmental conditions.

Variations in the carotenoid contents and the vitamin A potencies of six leafy vegetables were studied during their growth. The leafy vegetables studied include cauliflower, fenugreek, carrot leaves, rapeseed leaves, spinach and lettuce. The carotenoid contents as well as the vitamin A potencies were greater during the earlier stages of growth than when they reached the stage of maturity. All the vegetables except lettuce showed a steady increase in the carotenoid content during the earlier stages of growth after which there was a decline both in xanthophyll and carotene values throughout the growth period.

(iii) *Large-scale production of carotene.*—Large-scale production of carotene has been generally carried out by the extraction of dried plant materials with organic solvents and subsequent fractionation of the pigments. On account of large quantities of organic solvents required for the preparation, the alkaline digestion of the fresh plant material has been suggested as a preliminary to solvent extraction. Both these processes are unsuited for many reasons. Recent observations of Barnett that carotene can be elutriated by forcing jets of water and the elutriated carotene could be coagulated gives indications of a more practical method of preparation. Preliminary experiments are being conducted to find out the best means of decomposition of cell wall material to concentrate the carotene in the elutriate thus avoiding the use of large quantities of organic solvents. During the course of this year, one paper has been published and other seven papers have been communicated or are in the course of publication.

9. Enquiry on the growth-promoting factor present in Bengal gram under Professor M. Damodaran at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

In previous work on the effect of Biochanin C as a growth factor it was reported that when albino rats were given a daily dose of 150γ Biochanin C as a supplement to an otherwise adequate basal diet, the average weekly gain in weight was raised from 10 g. to 14 g. It was also found that a similar improvement could be obtained with a daily supplement of 3γ of thiamine chloride. Further experiments were carried out to confirm these findings.

In one experiment 4 groups of rats were taken with 6 animals in each group. The basal diet consisted of the following ingredients :—

	Per cent.
Starch	74
Casein	12
Yeast	4
McCollum's salt mixture	5
Fat	5

In addition each rat received cod-liver oil 1 drop, vitamin B_2 2-γ, pyridoxin hydrochloride 10 and calcium pantothenate 10γ. The experiment was run for 8 weeks and the weekly increase in weight recorded. The first group received the above basal diet; the second group received a supplement of 0·3 g. Bengal gram germ; the third group received a supplement of 3γ vitamin B_1 and the fourth group was given 150γ Biochanin C each rat per day. The results of this experiment are tabulated below :—

Group. number.	M.	F.	Total number of animals.	Diet.	Total growth for 8 weeks in grammes.
I	4	2	6	Basal diet ...	53 ±3·46
II	4	2	6	Basal diet+Bengal gram germ 0·3 g. ...	90·83±5·01
III	4	2	6	Basal diet+vitamin B_1 3γ	97 ±2·75
IV	4	2	6	Basal diet+Biochanin C 150γ ...	107 ±5·0

In the second experiment two basal diets were used. Diet A : Starch 65 per cent, casein 18 per cent, sugar 5 per cent, fat 7 per cent, salts 5 per cent. Diet B : Whole-wheat flour 64 parts, yeast 1 part, sodium chloride 1 part, calcium carbonate 1 part. This diet was supplemented with 40 c.c. milk each rat a day. The proximate chemical composition of the diets are given below :—

	Diet A, per cent.	Diet B, per cent.
Protein	19·0	19·0
Carbohydrate ...	73·5	71·5
Fat	7·5	9·5

Each animal received a supplement of the following vitamins:—
Vitamins A and D (as Adexolin) 2 drops on alternate days.

Vitamin E	...	3 mg. per week.
Vitamin B_1	...	3γ per day.
Vitamin B_2	...	2γ per day.
Calcium panthothenate	...	10γ per day.
Pyridoxin hydrochloride	..	10γ per day.

Group I received diet A only.
Group II received diet A + 150γ Biochanin C each rat per day.
Group III received diet B only.
Group IV received diet B + 150γ C Biochanin C each rat per day.
The results of the experiment are given below:—

Group number.	M.	F.	Total number of animals.	Diet.	Total increase in weight for 12 weeks.
I	3	3	6	A ...	159 ± 3·3
II	3	3	6	A + Biochanin C 150γ ...	174 ± 5·3
III	3	3	6	B ...	145 ± 5·3
IV	3	3	6	B + Biochanin C 150γ ...	173 ± 3·5

It can be concluded from these experiments that Biochanin C has a definite growth-promoting activity and that the improvement in growth produced by it is over and above the effect of vitamin B_1.

10. Enquiry on the metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta.

1. The work was started in May 1948 and the present short report deals with the work accomplished up-to-date. Some difficulty was experienced in obtaining a few essential chemicals for the estimation of the purine bases separately in urine. A definite advance has, however, been made during the four months' work.

2. Attempts were made to estimate the uric-acid excretion in urine of normal healthy individuals and of cases of tuberculosis. It was soon realized that the colorimetric method of Benedict (*Jour. Biol. Chem.*, 1922, 51, p. 187) using arseno-phospho-tungstic acid reagent was not suitable for our work as the colour development was achieved with other substances such as glucose and ascorbic acid present in pathological specimens of urine.

3. A suitable method was adopted following Kruger-Schmid's idea (1946, Hawk, Oser and Summerson's 'Practical Physiological Chemistry'), by which uric acid and other purine bodies were separated as copper complex from other constituents in urine. It was found that lithium-arseno-tungstate eliminated the greenish tinge observed with the use of Benedict's reagent. Control experiments according to the present method and the amount of recovery of added uric acid to pathological specimens of urine gave satisfactory results. The recovery was of the order of 94·6 per cent.

4. For the estimation of other purine bodies Hitchings' (*Jour. Biol. Chem.*, 1941, 140, p. 498) method was adopted. By this method xanthine and guanine were estimated together. Adenine and hypo-xanthine were not detectable in urine by this method.

5. Nucleic acid was prepared from dried yeast following the standard procedure, by extraction with dilute alkali, followed by acidification and precipitation by alcohol. This will be used for studying the behaviour of vitamin A towards different solutions of nucleic acid.

6. At present, investigations are being carried out with samples of urine from cases of (1) pulmonary tuberculosis, (2) acute nephritis and (3) leukæmia. There were no suitable cases of lobar pneumonia (in which condition the excretion of vitamin A is most constant) in the hospital wards for investigation. It is anticipated that some cases may be available for study during the next quarter.

7. In twenty-one samples of urine from tubercular patients the uric-acid excretion was constantly high ; varied between 71 mg. and 115 mg. per 100 c.c. with an average value of 77 mg. per 100 c.c. In one case detectable amounts of vitamin A were present. Unfortunately, it was not possible to keep track of this case because he was an out-patient in the hopsital and did not turn up again.

8. The result in a case of leukæmia investigated is very interesting. Urine of this case did not contain any protein. The uric-acid content was higher than in normal individuals (750 mg. per day). On shaking this urine with the non-saponifiable fraction of shark-liver oil, detectable amount of vitamin A was taken up by the urine. In previous work (Lawrie, Moore and Rajagopal, *Biochem. Jour.*, 1941, 7, p. 825) it was stated that urine which contained protein and vitamin A took up further amounts of vitamin A on shaking with vitamin A concentrate, but not when only protein was formerly present in urine. The present finding shows that even in the absence of protein in urine, but with significantly high amount of purines (uric acid) vitamin A goes into solution in the urine sample. This is a significant finding giving strength to the hypothesis that vitamin A and purine are in some combination in water-soluble form. Experiments are in progress to examine this point more thoroughly.

9. In acute nephritis the presence of vitamin A in urine is irregular. The urine contains high amounts of proteins and relatively small amounts of uric acid. Besides vitamin A a deep rosy-red colour is obtained in ethereal extracts. Attempts are made to characterize the nature of this colouring matter by spectroscopic tests.

11. Enquiry on the rôle of fat in lactose metabolism under Dr. S. Banerjee at the School of Tropical Medicine, Calcutta.

Excretion of galactose by skimmed-milk-fed rats.—Healthy rats of weights varying between 60 g. and 100 g. were fed skimmed milk for one month. They received a weekly supplement of a concentrate of vitamins A, D, E and K. The skimmed milk was mineralized with copper, manganese and iron. The animals were placed in metabolism cages and given a measured quantity of milk. The urinary excretion of galactose was estimated. Results are shown in Table I :—

TABLE I.

Twenty-four hours' urinary excretion of galactose (average of 20 rats).

Galactose ingested, g.	Galactose excreted, g.	Excretion × 100 / Ingestion.
2·41	0·264	11·0

The young rats excreted less amount of galactose in their urine. The mechanism of the utilization of galactose in young and old rats might be different.

When the skimmed-milk-fed rats were fed 2 c.c. of olive oil per day they stopped excreting galactose.

Estimation of liver glycogen in skimmed-milk-fed and whole-milk-fed rats.—Four groups of rats were placed on (1) skimmed milk, (2) whole milk, (3) skimmed milk with 2 c.c. of olive oil per animal per day, and skimmed milk with 5 g. of glucose per rat per day. They received the diet for one month. After this period all the animals were fed iso-caloric diet for two days and after a night's fast all the animals were killed by a blow on their head and the glycogen value of the liver was estimated. The results are given in Table II :—

TABLE II.

Glycogen value of the liver of rats.	Liver glycogen in mg. per 100 g. liver.
Skimmed-milk-fed rats (average of 3 rats)	68
Whole-milk-fed rats (average of 3 rats)	278
Skimmed-milk-fed rats receiving olive oil (average of 3 rats)	750
Skimmed-milk-fed rats receiving glucose (average of 3 rats)	2033

The above results indicate that the skimmed-milk-fed rats cannot store glycogen in their liver like the whole-milk-fed rats. It seems that liver cannot convert the galactose portion of the lactose ingested into glycogen in the absence of fat. The liver function of the skimmed-milk-fed rats is under investigation.

A method of estimation of galactose in blood :—A method of estimation of galactose in blood depending upon the fermentable oxidation of glucose in blood with *S. cerevisiae* followed by de-protenization and determination of the ferricyanide reducing power of the resulting filtrate has been developed.

Washed yeast is placed in a centrifuge tube; 2 c.c. of a 3 per cent sodium-sulphate solution of pH 4·4 is added to the tube. After addition of 0·2 c.c. of blood in the tube it is placed in an incubator at 37° C. for 15 minutes. 0·4 c.c. of 0·3N barium-hydroxide and 0·4 c.c. of a 5 per cent zinc-sulphate solutions are then added and thoroughly mixed. The mixture is centrifuged and to an aliquot portion of the centrifugate 2 c.c. of alkaline ferricyanide (0·005N) was added. The mixture was heated in a boiling water-bath for 15 minutes. The ferricyanide reduced was determined by titration with 0·005N sodium thiosulphate. 2 c.c. of 0·005N ferricyanide is reduced by 0·446 mg. of galactose.

List of papers published.

1. DAMODARAN, M., and YESHODA, K.—Amino Acids and Proteins in Hæmoglobin Formation. (*Biochem. Jour.*, 1947).
2. MISRA, U. C., and PATWARDHAN, V. N.—The Nutritive Value of Hydrogenated Oils. The Digestibility of Ground-nut (*Arachis hypogea*) Oil Hydrogenated to different Degrees of Hardness. (*I.J.M.R.*, **36**, No. 1 p. 27).
3. MUKUNDAN, R., and RAMA SASTRI, B. V.—A Simple Method for the Removal of Interfering Substances in the Estimation of Thiamine in Urine. (*I.J.M.R.*, 1948, **36**, No. 4).
4. PATWARDHAN, V. N.—Mode of Action of Vitamin D—A résumé. (*Curr. Sci.*, 1948, **17**).
5. PATWARDHAN V. N.—The Nutritive Value of Hydrogenated Oils (Vanaspati). (*Jour. Sci. & Ind. Res.*, 1948, **7**).
6. PATWARDHAN, V. N., and RAMALINGASWAMI, V.—Nutritional Deficiency Diseases in India with Demonstration of Cases. (*Jour. Christ. Med. Assn.*, July 1948).
7. RAMALINGASWAMI, V., and PATWARDHAN, V. N.—Diet and Health of the South Indian Plantation Labour. ((*I.J.M.R.* Jany. 1949).
8. RAMALINGASWAMI, V., VENKATACHALAM, P. S. and MENON, P. S.—Infantile pellagra : A Report of Five Cases. (*Indian Physician*, Sept. 1948).
9. RAMALINGASWAMI, V.—Nutritional Diarrhœa in Vitamin. A Deficiency. (*Ind. Jour. Med. Sci.*, Nov. 1948).
10. Observations on the Carotenoid Pigments of Different Varieties of Oranges. (*Jour. Sci. Ind. Res.*, 1948).
11. CHITRE, R. G., and DESAI, D. B.—Some Rich Sources of Active Carotenoids. (*Jour. Sci. Ind. Res.*, **6**, 1947).
12. CHITRE, R. G., DESAI, D. B., and BHARANI, S. P.—Nutritive Value of some Pure Strains of Cereals and Pulses. (*Ind. Jour. Med. Sci.*, 1948, **2**).

Report of the Seventeenth Meeting of the Nutrition Advisory Committee of the Indian Research Fund Association, held in the Senate House, Madras, on the 1st December, 1948.

PRESENT.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta. (*Chairman*).

Professor S. P. Agharkar, Head of the Department of Botany, Maharashtra Association for the Cultivation of Science, Law College, Poona 4.

Dr. S. Banerji, Head of the Department of Pysiology, Presidency College, Calcutta.

Dr. K. P. Basu, Dairy Chemist, Indian Dairy Research Institute, Bangalore.

Professor M. Damodaran, Assistant Director (Biochemistry), Council of Scientific & Industrial Research, University Buildings, Delhi.

Dr. B. C. Guha, Head of the Department of Applied Chemistry, University College of Science and Technology, 92, Upper Circular Road, Calcutta.

Dr. (Miss) R. Karnad, Deputy Director (Nutrition), Government of India, Ministry of Food, New Delhi.

Dr. R. B. Lal, Professor of Epidemiology of Malaria, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. K. Mitra, Adviser on Nutrition, Directorate-General of Health Services, New Delhi.

Professor V. Subrahmanyan, Planning Officer, Food Technological Research Institute, Council of Scientific & Industrial Research, Delhi.

Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. (*Secretary*).

Dr. C. O. Karunakaran and Mr. B. N. Adarkar did not attend.

The following were also present :—

Dr. D. V. Karmarkar, Technical Adviser, Ministry of Food, Government of India, New Delhi.

Mr. S. S. Pillai, Superintendent, Public Health Laboratory, Trivandrum.

Dr. K. C. Sen, Director, Indian Dairy Research Institute, Bangalore.

Dr. M. V. Radhakrishna Rao, Assistant Director, Haffkine Institute, Bombay.

Dr. Peng Cheng Hsu, F.A.O. Nutrition Division, Washington.

Lieut.-Colonel. M. L. Ahuja, Director, Central Research Institute, Kasauli.

Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

Lieut.-Colonel Barkat Narain, Director of Health Services, Delhi Province.

Miss E. D. Mason, Principal, Women's Christian College, Madras.

Miss D. M. Pearson, Professor of Nutrition, Women's Christian College, Madras.

Dr. M. B. Daver, Assistant Director of Public Health (Nutrition), Hyderabad.

Dr. B. P. Gyani, Nutrition Officer, Bihar.

Professor R. Subramaniam, Professor of Hygiene, Madras Medical College, Madras.

Dr. D. Subba Rao, Professor of Hygiene, Andhra Medical College, Vizagapatam, Nutrition Officer designate, Madras.

Dr. T. Lakshminarayana, Assistant Director of Public Health, Madras.

Dr. P. V. Gharpure, Professor of Pathology, Grant Medical College, Bombay.

Lieut.-Colonel O. P. Verma, Officer Commanding, Central Composite Food Laboratory, Army H. Q. (I.), New Delhi.

Dr. V. Ramalingaswami, Assistant Research Officer (Clinical Research), Nutrition Research Laboratories, Coonoor.

Dr. A. C. Barbara, Provincial Nutrition Officer, Assam.

Dr. S. Pandit, Adviser on Maternity & Child Welfare, Directorate-General of Health Services, New Delhi.

Dr. R. K. Anderson, Field Director in India, Rockefeller Foundation.

Dr. N. Veeraraghavan, Director, Pasteur Institute, Coonoor.

In making the following recommendations, the Committee considered the budgets very carefully and in view of the stringent financial condition suggested certain economies in the budgets proposed for the old and new enquiries to which the members unanimously agreed. Hence, no system of priorities was adopted in recommending the continuation of old and adoption of new enquiries.

1. To review work in progress and consider the question of continuation or otherwise of existing enquiries.

(A) The Committee recommended the continuation of the following enquiries :—

(i) Nutrition researches under Dr. V. N. Patwardhan at the Nutrition Research Laboratories, I.R.F.A., Coonoor.

(ii) Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

Dr. Guha is now a member of the Damodar Valley Corporation. He, however, gave the assurance to the Committee that, so long as he is in Calcutta, he will be able to supervise the work of the unit; the Laboratories at the University College of Science & Technology continue to be at his disposal.

(iii) Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

The Committee recommended that more concentrated effort to provide further information on the following problems should be made:—

(a) Nutritive value of pure-bred strains of cereals and pulses.

(b) The precursor and the physiological availability of nicotinic acid in foodstuffs.

(c) Absorption of fat from the intestines.

(iv) Enquiry on the rôle of fat in lactose metabolism under Dr. S. Banerji, Diabetes Research Worker, School of Tropical Medicine, Calcutta.

(v) Enquiry into metabolism of vitamin A (excretion of vitamin A in urine) under Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

(vi) Enquiry on the 'preparation of anti-rachitic substances' under Prof. B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory, S. P. College, Poona.

(vii) Bangalore school feeding scheme.

The Committee noted that the work on this scheme was to be completed by the end of December, 1948. The Committee also took note of the fact that Dr. Subrahmanyan is on duty outside the Indian Institute of Science, Bangalore, and his future plans are uncertain at the moment. The Committee had recommended last year that the staff engaged on soya-bean enquiry be absorbed in the nutrition research unit which may start in 1949-50 with a programme of research to be approved by the Committee. In view of the uncertainty about Dr. Subrahmanyan's future plans, the Committee recommended that the minimum staff be retained at this Unit with the budget of Rs. 7,954 for one year at the end of which it is expected that Dr. Subrahmanyan's plans will be finalized. The question of a full-fledged unit may be considered later.

(B) The following enquiries terminate at the end of the financial year.

(i) Enquiry on the formation, function and variations of plasma-proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

(ii) Enquiry on the evaluation of proteins in terms of hæmopoietic activity and the relation of dietary protein to blood and muscle hæmoglobin under Professor M. Damodaran at the Laboratories of the Council of Scientific and Industrial Research, Delhi.

(iii) Enquiry on plant carotenoids in relation to their biological importance under Dr. M. Damodaran at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

(iv) Enquiry on the growth-promoting factor present in Bengal gram under Dr. M. Damodaran at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

2. To consider new proposals.

(A) The Committee recommended the following new enquiries:—

(i) Enquiry into the nutritive value of foods to hospital patients in India under Dr. K. Mitra, Adviser on Nutrition, Directorate-General of Health Services, Government of India, New Delhi.

(ii) Enquiry into the effects of malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentrate as also the treatment thereof with high protein diets, proteins hydrolysates, etc. under Professor (Miss) S. Chowdhury and Dr. K. Mitra at the Lady Hardinge Medical College, New Delhi.

(iii) Enquiry on the studies on the bio-synthesis of nicotinic acid under Dr. S. Banerji, Head of the Department of Physiology, Presidency College, Calcutta.

(iv) Enquiry on the physiological effect on dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona.

The original proposal was for the establishment of a nutrition research unit. The Committee, however, recommended that the term "enquiry" should be used instead.

(B) The following enquiries were not recommended.

(i) Investigations into the causes of rancidity of fats with special reference to degradation by moulds under Prof. M. N. Goswami, Department of Applied Chemistry, University College of Science & Technology, Calcutta.

(ii) Enquiry on the study of the digestibility of various cooked Indian food preparations under Dr. J. D. Pathak, Topiawala National Medical College, Bombay.

(iii) Enquiry on multi-purpose food by Dr. N. Purshottam.

3. To consider the progress of nutrition work in provinces.

1. *Assam.*

Diet surveys were carried out on college hostel students, tea-garden labourers and in a tribal village. Multi-vitamin tablets were distributed through maternity and child welfare centres for the vulnerable groups in certain regions of the province. Education and publicity are being undertaken through the medium of lectures and distribution of informative pamphlets.

2. *Bihar.*

Diet surveys in the districts of Patna, Monghyr and Manbhum were carried out during the year. There had been outbreaks of lathyrism in these districts and hence the diets of villagers suffering from lathyrism and of those not affected with the disease were carried out in the same localities. The diets were generally low in calories and deficient in protective foodstuffs. Examination of over 3,000 school children was also carried out in different districts of the province. Other activities consisted of publicity and propaganda by means of exhibitions, talks and distribution of information.

3. *Bombay.*

Diet surveys were undertaken in the hospitals and messes in Bombay City and suburbs and thirteen other towns in Bombay Province and recommendations for improving the diets were made. In Bombay City, 718 school children were examined for their nutritional status; of these, 234 boys showed clinical signs of deficiency disease. The investigation on school feeding of 4,000 Parsi school children which commenced last year has been continued. Measures for increasing production of milk, fish, shark-liver oil, vitamin tablets, vegetables etc., are being carried out with vigour. Education and publicity campaigns are being pursued as in previous years.

4. *Madras.*

Diet surveys in eight districts of the province were carried out. The diets of the poor class people showed no appreciable variation from those

obtaining in the last few years. Nutrition surveys on 2,118 school children in some of these districts showed prevalence of deficiency disease due to lack of vitamins A, B complex and C.

The ameliorative measures consisted in the supply of free mid-day meals, milk, shark-liver oil, calcium lactate, yeast and multi-vitamin tablets in certain areas. Educational and publicity work has been done as in previous years.

4. To consider whether the recommendation of the Nutrition Advisory Committee, "that the fullest possible use of ground-nut cake flour should be made provided it is prepared under controlled conditions and every care is taken to prevent rancidity" should be modified (copy of a letter No. B.C. 2082/36/N.A.C., dated the 15th January, 1947, from Professor V. Subrahmanyan, D.Sc., F.R.I.C., Professor of Biochemistry, Indian Institute of Science, Bangalore, to the Secretary, Indian Research Fund Association, and of a letter No. 23/40/46-R. dated the 12th February, 1947, from the Secretary, Indian Research Fund Association, to Professor V. Subrahmanyan).

The Committee recorded the letters.

5. To discuss the nutritive value of common Indian sweets.

The Committee recommended that Dr. K. P. Basu, Dairy Chemist, Indian Dairy Research Institute, Bangalore, be requested to prepare a note outlining the scheme for investigation on the nutritive value of common Indian sweets. The note on preparation may be forwarded to the Secretary, Sugar Products Confectionery Panel, Department of Food, Government of India, New Delhi, by the Secretary, Indian Research Fund Association.

6. To note section 3(e) of the minutes of the sixth meeting of the Medical Research Liaison Committee for India held in India Office, London, on the 13th December, 1946.

Noted.

7. To consider the report on the adoption of uniform methods for the evaluation of nutritional status of population groups in India prepared by the Sub-Committee appointed by the Nutrition Advisory Committee at its meeting held on the 8th December, 1947 (Para 8 of the report of the sixteenth meeting of the Nutrition Advisory Committee—December, 1947).

The Committee adopted the report of the Sub-Committee attached as Appendix.

The Committee particularly wishes to draw the attention of nutrition officers in Provinces and States and in other institutions interested in the work to adopt the methods suggested in the report and report their findings at the next meeting of the Nutrition Advisory Committee.

8. To consider a request from the Secretary to the Government of Assam, Medical Department, Public Health Branch, Shillong, that the Indian Research Fund Association should undertake investigation into the effect of "white oil" on the human system.

As it was understood that the import of white oil in India has been banned recently, the problem of carrying out this investigation did not arise.

9. To consider the report of the Nutrition Committee of the F.A.O. of the United Nations held in Baguio, Philippines, from 23rd to the 28th February, 1948 on the nutrition problems of rice-eating countries in Asia and to note the statement made by Dr. V. N. Patwardhan, M.Sc., Ph.D., A.I.I.Sc., Director, Nutrition Research Laboratories, Coonoor and Chairman of the Committee while presenting the report at the F.A.O. Rice Meeting held in Baguio, Philippines, from 1st to 13th March, 1948.

Dr. Peng Cheng Hsu, Officer of the Nutrition Division of the F.A.O., Washington was present at the meeting and gave an account of the developments following the recommendations of the Baguio Nutrition Committee meeting.

The Committee recommended the postponement of this item for consideration at its next meeting.

10. To consider the possibility of publication of a popular monograph on wheat.

The Committee recommended that Dr. K. Mitra, Dr. K. P. Basu, and Dr. K. Rajagopal be requested to prepare the draft of the popular monograph and submit it for consideration at the next meeting of the Nutrition Advisory Committee. Dr. Peng Cheng Hsu stated that the Nutrition Division of F.A.O. had collected considerable information on the subject and the Organization would be very glad to be of assistance if needed.

APPENDIX.

Report of the Sub-Committee on the Adoption of Uniform Methods for the Evaluation of Nutritional Status of Population Groups.

This Sub-Committee was appointed at the last meeting of the Nutrition Advisory Committee at New Delhi on 8th December, 1947 and consisted of the following :—

Dr. G. Sankaran. (*Convener*).
Dr. K. Mitra.
Dr. P. D. Bhave.
Dr. C. O. Karunakaran.
Dr. V. N. Patwardhan.

The following additional members were co-opted :—

Dr. K. Rajagopal.
Dr. M. V. Radhakrishna Rao.
Lieut.-Colonel O. P. Verma.

A meeting of the Sub-Committee was held on Sunday, the 28th November at 9-30 a.m. Dr. Bhave and Dr. Karunakaran could not attend. Various memoranda submitted by the members were considered at the meeting. Discussion elicited the viewpoint that the purpose for which nutritional surveys might be undertaken could be classified as follows :—

1. Rapid surveys for emergency purposes.
2. Routine surveys.
3. Nutritional surveys for scientific investigations.

Hence the Sub-Committee felt that one uniform method would not serve the needs of the variety of surveys. It was, therefore, decided to have three different schedules for purposes of nutrition assessment, as follows :—

1. Rapid nutritional survey.
2. Clinical nutritional survey.
3. Nutritional survey supported by laboratory investigations.

It was further agreed that Dr. K. Mitra and Dr. M. V. Radhakrishna Rao should prepare draft of the methods to be used in " rapid nutritional surveys " and that Dr. V. N. Patwardhan and Dr. K. Rajagopal should prepare a similar one on " nutritional surveys supported by laboratory investigations ".

The draft prepared by Dr. Sankaran, the Convener, was taken as the basis for clinical nutritional survey. Accordingly, the draft schedule (pp. 73-77) for the assessment of nutritional status was adopted with the key for the same.

The Sub-Committee recommends that this schedule be used in all future nutritional surveys undertaken in India. The assessment of the value of the method and the interpretation of results can be made when sufficient data have been gathered with the use of these schedules.

The drafts for the two other schedules will be considered at a later date.

Nutritional Assessment Schedule.

		1. Sex
		2. Age
		3. Height
		4. Weight
		5. Hip width (Intercristal)
I. General.		6. Appearance
II. Eyes.	(A) Conjunctiva	7. Xerosis
		8. Pigmentation
		9. Discharge
	(B) Cornea	10. Xerosis
		11. Vascularisation
	(C) Lids	12. Excoriation
		13. Folliculosis
		14. Angular Conjunctivitis
		15. Night-blindness
III. Mouth.	(A) Lips	16. Condition
	(B) Tongue	17. Colour
		18. Surface
	(C) Buccal mucosa	19. Condition
	(D) Gums	20. Condition
	(E) Teeth	21. Fluorosis
		22. Caries
IV. Hair.		23. Condition
	(A) General	24. Appearance
V. Skin.		25. Elasticity

	(B) Regional	26. Trunk
		27. Face
		28. Perineum
		29. Extremities
VI.	Adipose tissue	30. Quantity
VII.	Œdema	31. Distribution
VIII.	Bones	32. Condition
IX.	Heart	33. Size
X.	Alimentary system	34. Appetite
		35. Stools
XI.	Nervous system	36. Calf tenderness
		37. Paresthesia

Key to the Nutritional Assessment Schedule.

I. General.

1. Sex
2. Age
3. Height
4. Weight
5. Hip width (Intercristal)
6. Appearance
   - 0 Good
   - 1 Fair
   - 2 Poor
   - 3 Very Poor

II. Eyes. (A) Conjunctiva

7. Xérosis
   - 0 Absent, glistening and moist.
   - 1 Slightly dry on exposure for ½ minute, lack of lustre.
   - 2 Conjunctiva dry and wrinkled.
   - 3 Conjunctiva very dry and Bitot's Spots present.
8. Pigmentation
   - 0 Normal colour.
   - 1 Slight discolouration.
   - 2 Moderate browning in patches.
   - 3 Severe earthy discoloration.

9. Discharge
   - 0 Absent
   - 1 Watery, excessive lachrymation
   - 2 Mucopurulent
   - 3 Purulent

(B) Cornea

10. Xerosis
    - 0 Absent
    - 1 Slight dryness and diminished sensibility
    - 2 Haziness and diminished transparency
    - 3 Ulceration

11. Vascularisation
    - 0 Absent
    - 1 Circumcorneal injection of blood vessels
    - 2 Vascularisation of cornea

(C) Lids.

12. Excoriation
    - 0 Absent
    - 1. Slight excoriation
    - 2 Blepharitis

13. Folliculosis
    - 0 Absent
    - 1 A few granules
    - 2 Lids covered with extensive granules
    - 3 Hypertrophy

14. Angular conjunctivitis
    - 0 Absent
    - 1 Present

(D) Functional

15. Night-blindness
    - 0 Absent
    - 1 Present

    N.B. Exclude other eye diseases not associated with nutritional defects.

III. Mouth. (A) Lips

16. Condition
    - 0 Normal
    - 1 Angular stomatitis, mild
    - 2 Angular stomatitis, marked

(B) Tongue

17. Colour.
    - 0 Normal
    - 1 Pale but not coated
    - 2 Red
    - 3 Red and raw

18. Surface
    0 Normal
    1 Fissured
    2 Ulcered
    3 Glazed and atrophic

(C) Buccal mucosa

19. Condition
    0 Normal
    1 Stomatitis

(D) Gums

20. Condition
    0 Normal
    1 Bleeding and /or gingivitis
    2 Pyorrhœa
    3 Retracted

(E) Teeth

21. Fluorosis
    0 Absent
    1 Chalky teeth
    2 Pitting of teeth
    3 Mottled and discoloured teeth

22. Caries
    0 Absent
    1 Slight
    2 Marked

IV. Hair.

23. Condition
    0 Normal
    1 Loss of lustre
    2 Discoloured and dry
    3 Sparse and brittle

V. Skin (A) General

24. Appearance
    0 Normal
    1 Loss of lustre
    2 Dry and rough or crazy pavement
    3 Hyperkeratosis, phrynoderma

25. Elasticity
    0 Normal
    1 Diminished
    2 Wrinkled skin

(B) Regional

26. Trunk
    0 Normal
    1 Collar-like pigmentation and dermatitis around the neck

27. Face
    0 Normal
    1 Nasolabial seborrhœa
    2 Symmetrical sub-orbital pigmentation

28. Perineum
    0 Normal
    1 Scrotal or pudendal dermatitis
29. Extremities
    0 Normal
    1 Symmetrical dermatitis with pigmentation of glove or stocking type

VI. Adipose tissue. (to be judged by the examination of the arm over the biceps)

30. Quantity
    0 Normal
    1 Deficient

VII. Œdema.

31. Distribution
    0 Absent
    1 Œdema on dependent parts
    2 Œdema on face and dependent parts
    3 General anasarca

VIII. Bones.

32. Condition.
    0 Normal
    1 Stigmata of past rickets

IX. Heart.

33. Size.
    0 Normal
    1 Apex just outside the nipple line
    2 Enlarged

X. Alimentary system.

34. Appetite
    0 Normal
    1 Anorexia
35. Stools.
    0 Normal evacuation
    1 Diarrhœa

XI. Nervous system.

36. Calf tenderness
    0 Absent
    1 Present
37. Paresthesia
    0 Absent
    1 Present

LEPROSY.

1. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

The work during the year included (i) an investigation of the therapeutic value of the sulphone drugs in leprosy, (ii) investigation whether cockroaches transmit leprosy, (iii) a study of lesions of leprosy in ear, nose and throat, (iv) a study of histopathology of selected cases of leprosy.

The sulphone drugs in leprosy.

The sulphone drugs are derivatives of di-amino-di-phenyl-sulphone. They include promin, diasone and sulphetrone. These medicines were tried on some lepromatous cases of leprosy.

Selection of cases.—Sixty-four lepromatous cases of Albert Victor Hospital (Calcutta) were selected for special treatment with sulphone drugs. Fourteen patients were kept under promin, twenty under diasone and thirty under sulphetrone. All the patients were bacteriologically positive and lepromin negative. Several of them were suffering from leprotic ulcers, iritis, repeated reactions etc. Some of the patients could not stand even small doses of hydnocarpus oil.

Preliminary steps.—Before starting the treatment, blood examination was made in each case to find out the total counts of r.b.c. and w.b.c. percentage of haemoglobin and cell volume. If there was anaemia, the patient was first put on a course of iron and liver extract until the blood picture improved. Vitamin B was given to all cases. All the patients received a supplementary diet in the form of increased amount of milk and meat.

Dosage.—The initial dose of promin was 1 g. and it was gradually increased upto 5 g. in some cases, but the majority could not stand more than 2 g. to 3 g. Promin was given intravenously. Diasone and sulphetrone were given per mouth. The dose of diasone was $\frac{1}{2}$ g. daily to begin with. It was increased to $1\frac{1}{2}$ g. per day in most cases and 2 g. in some cases. The starting dose of sulphetrone was $1\frac{1}{2}$ g. per day and it was gradually increased to 6 g. in most cases. The treatment was given for six days in a week, and after two weeks' treatment rest was given for one week. Later treatment was given for 2 months after which there was rest for 1 month.

Blood findings.—Blood examinations were done for two purposes: (a) for detection of anaemia due to the administration of sulphone drugs, (b) to find out the concentration of sulphone drugs in the blood.

In almost all cases the administration of the drugs gave rise to lower red cell count and hæmoglobin values, but in most cases the blood picture could be improved and maintained at a fair level by the administration of iron with or without liver.

Estimations of sulphone drugs in the blood were made periodically to regulate the dosage. The findings are summarized below. On the basis of these findings a paper has been submitted to the International Leprosy Congress of 1948.

When promin was given intravenously in 4 g. to 5 g. doses the concentration of the drug in the blood was usually 10 mg. to 12 mg. per 100 c.c. about one

hour after injection. The drug disappeared completely from the blood after about 12 hours. The diasone and sulphetrone were given per mouth. The highest concentrations of these drugs, in the blood, was seen after four hours and the drug usually disappeared from the blood after 24 hours. But in one or two cases the drug persisted longer. With both these drugs the average blood concentration was about 2 mg. per 100 c.c. and the maximum was 3 mg. per 100 c.c. But this tended to fall after the patient remained under treatment for some time, when the blood concentration was as low as 0·5 g. per 100 c.c. although the dose of the medicine was same as before. When these drugs were given continuously for two months and when the rest period after that was extended up to 1 month, it was found that after the rest period the concentration of these drugs tended to rise gradually to near about the original level.

The *in vitro* estimation of the three sulphone drugs were made by the method of Marshall and that of Burroughs Wellcome and the results were compared. This was done by taking known amounts of the above drugs in known volume of blood from an untreated lepromatous case. It was found that the method of Burroughs Wellcome gave uniformly better results than the Marshall method. The superiority of the former method was more marked in the case of sulphetrone and diasone than in the case of promin and also when the concentration of the drugs in the sample was high, i.e. 10 mg. per 100 c.c.

Therapeutic results.—The promin group of patients are now under treatment for about 2 years, the diasone group for 1½ years and sulphetrone group for 1 year. Some of the cases could not stand promin, and they were placed under diasone or sulphetrone.

Clinical improvement had been seen in most of the cases under these groups, excepting in a small number who could not tolerate adequate doses. The improvement consisted of subsidence of areas of infiltration, disappearance of small nodules, decrease in size and softening of big nodules and improvement in the eye conditions and subsidence of inflamed glands. The most marked results had been seen in the treatment of ulcers, both general and nasal, which healed up quickly and did not recur. Tendency to reaction was checked or minimized, the reactions not recurring or recurring less frequently and with less intensity.

Bacteriological improvement had been noticed but they were not so marked as clinical improvements. It had been reported by other leprosy workers that patients became negative after prolonged treatment.

The treatment had certain limitations. It is costly. The drugs had no effect on the nerve pains and nerve abscesses, which were marked features in some of the cases under treatment. Other neural symptoms such as loss of sensation and deformities did not show any improvement.

Transmission of leprosy by cockroaches.

It was reported by Moiser (Africa) that cockroaches transmit leprosy. With a view to eliciting this point an investigation was undertaken. The findings and conclusions of this investigation, which are given below, have been presented in a paper and submitted at the International Leprosy Congress of 1948 :—

Cockroaches were collected from endemic and non-endemic areas. Acid-fast bacilli were found in the guts of roaches from both these areas. They could be easily cultivated on laboratory media. Feeding experiments showed that leprosy bacilli ingested by cockroaches did not multiply in their guts. From all these it is concluded that cockroaches do not play any important rôle in the transmission of leprosy. Like domestic flies they may, however, be mechanical carriers.

STUDY OF NOSE AND THROAT LESIONS IN LEPROSY.

(a) An investigation had been made of the frequency with which leprosy bacilli could be found in nasal mucosa in cases of leprosy of different types, with a special reference to the neural cases in which smears from skin lesions were negative for acid-fast bacilli.

The total number of cases examined were 4,072. Multiple smears from skin lesions and from the nasal mucous membrane were examined from all cases and the findings are tabulated below:—

Type.	Skin smears.	Total number of cases.	Number of cases in which nasal smear was negative.	Number of cases in which nasal smear was positive.	Percentage of cases in which nasal smear was positive. %
Neural (N) ...	Negative	2,834	2,829	5	0·17
Neural (N) ...	Positive	447	289	158	35·4
Doubtful (N? L) ...	Positive	121	34	87	72·0
Lepromatous (L) ...	Positive	670	51	619	92·4

Conclusions.—The conclusions derived from the investigation are the following:—

(1) In case of leprosy of all types the bacilli are found more frequently in the skin lesions than in the nasal smears. This difference is specially marked in cases of the neural type.

(2) In cases of the neural type with bacteriologically negative skin lesions leprosy bacilli are seldom found in the nasal mucosa. In only 0·17 per cent of neural cases the nasal mucosa was positive when the skin smear was negative.

(3) Although the finding of leprosy bacilli in the nasal mucosa, in the absence of bacilli in the skin smear, is not a common feature, it is important to make a bacteriological examination of the nasal mucous membrane in addition to the examination of skin smears before declaring a patient "closed" or non-infectious case.

(4) The results of the investigation do not support the contention of some workers that nose is the portal of entry of leprosy bacilli.

(b) The other line of investigation was to find out leprous lesions in the ear, nose and throat of leprosy cases. This investigation had been started only recently, in collaboration with Major K. N. Ghosh, M.B., F.R.C.S., D.L.O., of the Calcutta Medical College.

So far 17 cases had been examined of whom 15 were lepromatous and 2 were neural cases.

The changes are specially marked in lepromatous cases and the results so far indicate that leprous lesions with infiltration, ulceration or atrophy of the mucous membrane are rather common in the nasal mucosa. Perforation

of the septum is also a common incidence and the part involved is usually found at the junction of the cartilagenous and bony portion. Atrophy of the inferior turbinate bone was also observed in a number of cases. Smears as usual show in all cases a large number of acid-fast bacilli with globi.

Involvement of the naso-pharyngeal, pharyngeal and laryngeal mucosa is not as frequent as the nasal mucosa. Bacilli have been found in four out of fifteen lepromatous cases. In one case pharyngeal node was enlarged and showed acid-fast bacilli. In another instance laryngeal mucosa over the epiglottis and tip of the artytenoid cartilage showed infiltration with formation of small nodules.

The study is continued.

Histopathological work.

Histopathological examinations of biopsy specimens from selected cases of leprosy had been continued. Several groups of cases were under this study. Repeated examinations had been made on each individual case and clinical, bacteriological, histological and immunological changes had been noted. The findings in two groups of cases of leprosy, viz. cases of doubtful classification and that of tuberculoid reaction have been analysed so far, and included in two papers and presented at the International Leprosy Congress, 1948.

(a) Cases of "doubtful" classification.

On clinical examination 31 cases were classified as "doubtful". The lesions in these cases resembled tuberculoid lesions being localized, and the lepromatous lesions being smooth and shiny with ill-defined margins and the smears showed moderate to large number of bacilli. After thorough clinical, bacteriological, histological and immunological examinations, it was possible to find out that out of 31 cases 18 were lepromatous and 3 tuberculoid. In the remaining 10 cases a proper classification was not possible even after repeated examinations and the classification of these remained "doubtful".

Conclusions.

(1) In the 18 cases ultimately classified as lepromatous, the disease was progressive and remained active for long. Amongst all these cases the disease later became generalized and the cases were then clinically lepromatous but in a small number the lesions always remained localized. The lepromin test was usually negative or doubtful throughout, the histological picture was that of a lepromatous lesion but the granuloma was focal in distribution and the foci tended to show tuberculoid arrangement. It appears that in the lepromatous type localized lesions occur more frequently than is generally recognized.

(2) In the 3 cases ultimately classified as tuberculoid, early clinical and bacteriological subsidence was seen. The lepromin test was positive, the histology was essentially tuberculoid, but there were present certain atypical features such as small and scanty giant cells, vacuolation of the epithelioid cells, non-infiltration of the sub-epithelioid cells, non-infiltration of the nerves and comparative freedom from infiltration of the sub-epithelial layer.

(3) In the 10 cases in which the classification still remained doubtful early clinical subsidence was usual, but it was often followed by relapse of activity and leprosy bacilli could often be found even after the clinical subsidence

of the disease. In the histological picture were present elements of both the tuberculoid and the lepromatous histology, but neither of them was marked enough for the section to be labelled as tuberculoid or lepromatous. The lepromin test was variable. In these cases the clinical, histological and immunological features appeared to be in an unstable state. Similar cases have been encountered in a study of another group of cases with what looked as reacting tuberculoid lesions.

(4) In sorting out the above groups, the lepromin test and histological examination are of definite help. In case of active lesions a positive lepromin test rules out lepromatous type and a negative lepromin test rules out tuberculoid type. Histological examination will confirm and at times further clear-up the classification but the results of the first examination may not be conclusive and subsequent biopsies may be needed. Moreover, it has to be clearly borne in mind that in the cases with lesions clinically not typical of either type, the histological picture has also certain atypical features which it is essential to recognize. Thus, both the lepromin test and the histological findings are important in the classification of such cases, but both have their limitations.

(5) Two cases are reported in which a change of type to lepromatous was seen during the period of observation. In the absence of a critical outlook this change could have been described as a change from tuberculoid to lepromatous which really it was not; actually the change took place in the lesions which simulated the tuberculoid lesions, but were not really so, as judged by the immunological and histological findings. The lepromin test was all along negative in these cases and the histological picture was never typically tuberculoid.

(b) Cases of tuberculoid reaction.

There were 33 cases under study. In this group were included tuberculoid cases in which the history of the disease definitely indicated the presence of reaction, mild or moderate, i.e. the disease had existed for some time, but the signs of exacerbation were present only for the previous few months. In some cases the lesions were typically tuberculoid with well-defined margins but in others lesions were ill-defined and smooth and had a shiny surface.

As a result of thorough clinical, bacteriological, histological and immunological examinations it was possible to divide the cases into three groups :—

(i) Twenty-one out of 33 were tuberculoid reaction cases.
(ii) Three were definitely lepromatous.
(iii) In the remaining 9 cases the findings were not clear-cut and, therefore, the classification remained " doubtful ".

Conclusions.

(1) The small lepromatous group showed that at times lepromatous lesions may be so localized as to simulate tuberculoid lesions. The distinction was easily made by means of the lepromin test, the result being negative in lepromatous cases and positive in tuberculoid cases, more so in the stage of reaction. The histology was not always typically lepromatous as in the midst of lepromatous histology some tuberculoid elements were seen in the form of foci of epithelioid cells surrounded by round cells and small giant cells.

(2) The study of tuberculoid group showed that the true tuberculoid histology was not always found. During the stage of reaction there were found such features as markedly vacuolated cells, small and scanty giant cells,

uninfiltrated nerves and relative freedom of sub-epithelial layer. In all these cases the lesions as a rule subsided early. The lepromin test was always positive. Smears from skin lesions were either negative or slightly positive, during reaction, but with the subsidence of reaction the bacilli disappeared.

(3) The cases of "doubtful" classification simulate the reacting tuberculoid cases on the clinical course, the bacteriological and histological findings and the results of the lepromin test is different from the true tuberculoid cases. In these cases early subsidence is commonly seen, but in most cases this is followed by repeated reactions. The lesions contain moderate to large number of bacilli and these persist for long even after the clinical subsidence. Lepromin test is positive during the increased activity, but with clinical subsidence it becomes negative or doubtful. In the histological picture are present both lepromatous and tuberculoid elements, but none of them are found to a marked extent, and the actual histological picture varies according to whether one or the other is more marked. Thus, in these cases all the features—clinical, histological, bacteriological and immunological—appear to be in an unstable state. These cases will claim a place in any system of classification of leprosy.

2. Enquiry on the histopathology of leprosy under Dr. R. G. Cochrane, Honorary Medical Superintendent, Government Lady Willingdon Leprosy Sanatorium, Chingleput.

Many cases of all types of leprosy were biopsied and certain fundamental pathological concepts are being built up. It was reported last year that the special staining for acid-fast bacilli in the tissue as used by Fite has been adopted. Since using this modification of standard acid-fast technique clearer light is being shed on the role of the *M. lepræ* in the tissues. It has been long held that there are four different histo-pathological pictures seen in leprosy :—

(1) Early round-celled infiltration.

(2) Focal infiltration consisting mainly of epithelioid and giant cells (tuberculoid).

(3) Diffuse infiltration of lepra cells (*Leproma*).

(4) An infiltration, very varied in type, associated with the intermediate lesions.

It appears that there is a fairly logical explanation of the pathology of leprosy, although at first the great variations in the type of cells mobilized almost suggest different pathological processes. This, however, is not the case, for the cellular reaction is probably due to the type of tissue response stimulated rather than to any specific toxic effect of *M. lepræ*. The *M. lepræ* appears to survive in the tissues most easily if the reticulo-endothelial system is invaded by the organism and a state of commensalism is set up between the bacilli and the cells of the reticulo-endothelial system.

It has been stated previously that it was considered that, as in tuberculosis so in leprosy, there is an initial focus (Ghon focus) and that this focus might be in the reticulo-endothelial system. This conception may be wrong for evidence is accumulating suggesting that the initial focus may be in the skin, possibly in that area of relative vascularity—the junction of the pars reticularis and the pars papillaris in the corium. It is known that there is at this point a rich net-work of lymphatics, capillaries and nerves, and that fine nerve-fibres and vessels pass into the rete from here. It is apparent that the first cell to be

mobilized, as would be expected in a low grade inflammatory process such as leprosy, is the round cell (lymphocyte). According to Cowdry the lymphocyte is the precursor, both of the epitheloid cell and of the histiocyte, and the macrophage a development from the histiocyte; the macrophage is an end product and, therefore, does not develop further, although it will undergo degeneration as seen in the foamy cell.

With the new technique of acid-fast staining it appears that the first demonstrable bacilli are found in the small nerves of the corium, in fact an observation which may be of some importance is that the less the cellular activity in these lesions the more likely are bacilli to be found in the small nerves.

It is suggested that the bacilli enter the skin and are first present—they are too few to be demonstrable—at the junction of the pars papillaris and pars reticularis of the corium. Lymphocytic infiltration is the first evidence of tissue reaction. Assuming the tissues are relatively inactive then the bacilli multiply and appear first in the nerves, in such instances the bacilli continue to multiply and they spread up the nerve sheath and also through the tissues of the corium. As the bacilli multiply so there is a more rapid evolution of the lymphocytes; these are transformed into histiocytes and develop into the phagocytic macrophage—the lepra cell. The line of spread is naturally outwards and downwards and so the granulomatous infiltrations are seen diffusely scattered under the epidermis, leaving a narrow relatively clear sub-epidermal zone. The bacilli are thus carried through lymphatics to the lymph nodes and thence to the spleen and liver, and the entire reticulo-endothelial system becomes parasitized by the organism, which, because of its low pathogenicity, behaves more like inert matter than an active pathogenic organism. It is suggested that it is in this way that lepromatous leprosy becomes progressive.

On the other hand, if there is potential tissue resistance, the evolution of the lesion is entirely different. In the early macule in a case with potential tissue resistance, round cells are mobilized and are concentrated in definite areas: hair-follicles, sweat-glands and vessels. As the bacilli or their products stimulate tissue defence, the lymphocyte develops, not into the histiocyte and macrophage, but into the epithelioid cell and giant cell and epithelioid and giant cell foci appear as large masses. These naturally are found at the points of concentration of the lymphocytes—hair-follicles, sweat-glands and vessels. It is tempting to suggest that the points of entry of the *M. lepræ* are the hair-follicles and the ducts of the sweat-glands. The greater the tissue response the more evidence will there be of an intense focalization of the granulomatous tissue. Because this is essentially a tissue anchoring process these tuberculoid masses are seen extending right up against the epidermis and there is no relatively free sub-epidermal zone. No bacilli are demonstrable, particularly when the tissue reaction is maximal, evidently because it only needs a few bacilli to stimulate the response, which appears to prevent their multiplication and ultimately, one assumes, causes death of the organism.

The two tissue reactions described are the two extremes or polar reactions which result in the two polar types of the South American Classification—tuberculoid and leproma. There appear, however, to be gradations between active tuberculoid, with a much more marked epithelioid cell reaction, and the so called intermediate lesion with a more succulent clinical picture and a diffuse pistribution of the epithelioid cell masses and a free sub-epidermal zone, to frank

leproma. There is a point, it is believed, when tuberculoid remains true to type. Whether tuberculoid leprosy ever shows a tendency to become transformed to leproma is not yet clear, but there is evidence that atypical lesions become leproma.

Finally, there appears a third type of tissue reaction, rare though it is, which has been described by early workers and which resulted in Muir's theory of an ascending nerve infection. Evidently, for reasons quite unknown, the bacilli, instead of multiplying in the skin, multiply and pass up the nerve sheath, this results in pure neural anæsthetic leprosy. This reaction occurs because the ascending nerve approach is apparently the line of least resistance, and is not a true manifestation of effective defence, because it is known that exceptionally these cases do become lepromatous.

It will be seen that the above preliminary conclusions open a most interesting line of investigation and hold promise of a definite advance in our basic pathological conception of the disease.

List of papers published.

DHARMENDRA.—Planning of Leprosy Work in India. All-India Leprosy Workers' Conference, Wardha.

DHARMENDRA.—Need for fresh Anti-leprosy Legislation in India. All-India Leprosy Workers' Conference, Wardha.

DHARMENDRA.—The Place of Vasectomy in Leprosy Control. All-India Leprosy Workers' Conference, Wardha.

DHARMENDRA.—Classification of Leprosy. All-India Leprosy Workers' Conference, Wardha.

DHARMENDRA and CHATTERJEE, K. R.—Treatment of Leprosy with the Sulphone Drugs. All-India Leprosy Workers' Conference, Wardha.

DHARMENDRA, MUKERJEE, N., and CHATTERJEE, S. N.—A Follow-up Study of Reacting Tuberculoid Lesions. International Leprosy Congress, Cuba.

DHARMENDRA, CHATTERJEE, S. N., and MUKERJEE, N.—A Follow-up Study of Leprosy Cases of 'Doubtful' Classification. International Leprosy Congress, Cuba.

DHARMENDRA and MUKERJEE, N.—Cockroaches not Responsible for Transmission of Leprosy. International Leprosy Congress, Cuba.

DHARMENDRA.—Treatment of Leprosy with Sulphone Drugs. International Leprosy Congress, Cuba.

DHARMENDRA.—Classification of Leprosy. International Leprosy Congress, Cuba.

DHARMENDRA and SEN, N. R.—Frequency of the Presence of Leprosy Bacilli in Nasal Smears of Leprosy Patients. International Leprosy Congress, Cuba.

DHARMENDRA and MUKERJEE, N.—Effect of Sunlight on the Staining Properties of Leprosy Bacilli. International Leprosy Congress, Cuba.

REPORT OF THE MEETING OF THE LEPROSY ADVISORY COMMITTEE.

Held at 2-30 p.m. on the 27th November, 1948 in the Senate House, Madras.

PRESENT.

Dr. C. G. Pandit. (*Chairman*).
Dr. R. G. Cochrane.
Dr. P. V. Gharpure.
Dr. V. R. Khanolkar.
Dr. Dharmendra. (*Secretary*).

IN ATTENDANCE.

Lieut.-Colonel B. N. Hajra.
Colonel C. S. P. Hamilton.
Dr. J. M. Mungavin.

(i) Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

(ii) Enquiry on the histopathology of leprosy under Dr. R. G. Cochrane at the Government Lady Willingdon Leprosy Sanatorium, Chingleput.

The reports received from Dr. Dharmendra and Dr. Cochrane regarding the progress of work done in their respective enquiries on leprosy were considered, and the continuance of these two enquiries for the year 1949-50 was recommended.

The Committee further decided that the budget of the enquiry under Dr. Dharmendra should not exceed Rs. 19,489/- and of that under Dr. Cochrane should not exceed Rs. 19,875/-.

Dr. Cochrane had originally provided a biochemist at Rs. 200/- p.m. in the scale Rs. 160-10-330 but it was considered that a suitable qualified biochemist may not be available at this pay, and the Committee, therefore, recommended that the scale of pay of the biochemist be revised to Rs. 275-25-500-E.B.-30-590. The budget passed for this enquiry includes the revised scale of pay.

Dr. Khanolkar stressed the need of obtaining leprosy material from countries other than India in order that the complete histological picture of the disease can be obtained, since there is evidence that there are wide variations in the types of disease in different countries. It was suggested that Dr. Khanolkar may submit a short note on the subject so that attempts may be made to obtain the necessary material from other countries.

Colonel Hamilton, Medical Officer, Juri Tea Estate, Sylhet and in-charge of Juri Valley Medical Association and Ross Institute Field Research Centre reported on the use of diasone in small doses in the treatment of outdoor patients of leprosy, both neurol and lepromatous, with beneficial results. The Chairman thanked Colonel Hamilton for the trouble he had taken to come all the way from Bengal to present his results to the Committee.

The Committee noted that the review of the lepromin test by Dr. Dharmendra was published as a special number by the British Empire Leprosy Relief Association, London.

The Committee decided that in view of certain recent developments, it was not necessary at present to proceed with a review of leprosy work in India.

The forms prepared by Dr. Cochrane for recording information regarding trials of chemotherapeutic compounds used in the treatment of leprosy were considered and the Committee recommended them for trial by workers in leprosy.

PLAGUE.

1. Plague researches under the Director, Haffkine Institute, Bombay.

I. Plague vaccine.

Following the experience of Dubes to produce submerged and also enhancement of growth by the addition of surface active agents to media, experiments were tried to enhance the growth of plague organisms, by the addition of a surface-active agent (tween 80) to the casein hydrolysate medium described in the earlier report. It was observed that the addition of a surface-active agent produced more abundant, less clumpy and consequently more uniformly suspended growth. This observation which is of great value in the production of plague vaccine was utilized to produce plague vaccine in this new medium with 0·01 per cent tween added to the casein hydrolysate medium. In order to make the plague vaccine almost free from clumps which has been a great handicap to the Haffkine plague vaccine, the temperature of incubation was raised to 32°C. This temperature just satisfies the purpose by giving abundant growth free from clumps. Higher temperatures reduce the growth yield and lower temperatures produce clumps.

Vaccines were prepared in media with and without tween and were tested for their mouse protective values. The following results were obtained :—

	50·0 per cent mouse protective dose, average of 3 experiments.
(1) Two weeks' vaccine grown at 32°C. killed with 0·07 per cent formalin and preserved with 1·5 mg. phenyl mercuric nitrate per 100 c.c. (medium with 0·01 per cent tween). ...	0·0031 c.c.
(2) Two weeks' vaccine grown at 32°C. killed with 0·07 per cent formalin and preserved with 1·5 mg. phenyl mercuric nitrate per 100 c.c. (medium without tween) ...	0·0051 c.c.

In later batches of vaccines, a mouse protective dose of about 0·0025 c.c. was consistently obtained.

II. Chemotherapy of plague.

Polymixin.—This new anti-biotic which is supposed to possess remarkable specificity for Gram-negative organisms, even superior to streptomycin in some cases, was tried for its protective value in experimental plague infection in mice. The following results were obtained :—

Drug given subcutaneously ; requisite quantity contained in 0·2 c.c. of the fluid.

Drug given in one dose only immediately after infection.

Dose.	Number of deaths in test animals.	Number of organisms in challenge dose.	Deaths in controls.
5 mg. in one dose ...	2/5	100	12/12
1·0 mg. in one dose ...	5/5	100	12/12
0·5 mg. in one dose ...	5/5	100	12/12
0·25 mg. in one dose ...	4/5	100	12/12

Drug administration started 48 hours after infection.

Dose.	Number of deaths in test animals.	Number of organisms in challenge dose.	Deaths in controls.	Remarks.
5 mg. four times daily at 6 hours' interval ...	5/5	128	10/10	The animals died within 18 hours after commencement of drug in both the sets. Evidently, the animals died of toxicity.
—Do.—	5/5	No test dose given	...	
1·0 mg. four times daily at 6 hours interval	10/10	128	10/10	All animals died after 8 injections. Animals died of toxicity.

III. FIELD TRIALS WITH STREPTOMYCIN, SULPHADIAZINE AND SULPHAMERAZINE.

A plague epidemic at Manchar in Poona district during the latter part of 1947 and early part of 1948 gave an opportunity to conduct a trial of the comparative curative value of streptomycin, sulphadiazine and sulphamerazine in the treatment of bubonic plague. The trial was carried out at the mobile plague hospital stationed at Manchar and lasted from 26th Novembr, 1947 to 22nd March, 1948. During this period 313 cases were admitted to hospital. Cases were treated in rotation with different treatments. Thus from case No. 1 to 112, cases were treated alternately with sulphadiazine and streptomycin, and from case 113 to 300 alternately with sulphamerazine and streptomycin. From 301 to 313, however, all cases were treated with streptomycin.

The drugs were given in the following dosage :—

Streptomycin.—In mild cases, an initial dose of 2/3 g. followed by 1/3 g. every 4 hours was given intramuscularly until the temperature remained normal for 24 hours. In severe cases, 2/3 g. of the drug every 4 hours was given until the temperature remained normal for 2 to 3 days. In a few cases in which the estimation was done the concentration of the drug reached 5 units in 4 hours, 10 units in 8 hours and 20 units or more after 24 hours per ml. of serum.

Sulphadiazine.—An initial dose of 4 g. was followed by 2 g. four hours later. Thereafter, one g. was given every 4 hours until the patient's temperature remained normal for 2 days. This dose maintained a concentration of 10 mg. to 15 mg. per 100 c.c. of blood.

Sulphamerazine.—An initial dose of 4 g. was followed by 1 g. 4 hours later. Then one g. was given every 8 hours till the temperature remained normal for 2 days. This dose maintained a concentration between 10 mg. to 20 mg. per 100 c.c. of blood.

Both the sulpha drugs were given orally as far as possible. Cases which were comatose or were unable to swallow the drugs for any reason received the drugs intravenously or intramuscularly. The drugs were given, if necessary, for a total period of 10 to 12 days.

Results.—Of the 313 cases admitted to hospital 287 cases subsequently turned out to be plague. From these, 36 cases have been excluded from reckoning: (1) 18 convalescent cases, (2) 14 moribund cases, (3) 4 receiving combined treatment of streptomycin and sulphamerazine or anti-plague serum.

The results obtained in the remaining 251 cases are summarized in the following tables:—

All cases.

Treatment.	Number of cases.	Number of deaths.	Case mortality, per cent.
Streptomycin ...	132	5	3·8
Sulphadiazine ...	17	2	4·2
Sulphamerazine ...	72	6	8·3

Since septicaemic cases gave a better idea of the curative power of any treatment, the results of the septicaemic cases are given below separately:—

Treatment.	Number of cases.	Number of deaths.	Case mortality, per cent.
Streptomycin ...	32	3	9·3
Sulphadiazine ...	11	2	18·1
Sulphamerazine ...	13	5	38·4

The results obtained with different treatments may not show statistically significant differences, but the effect of streptomycin was striking. Both in non-septicaemic as well as in septicaemic cases, the temperature in cases treated with streptomycin came to normal earlier than that in cases treated with either of the sulpha drugs. Thus, in septicaemic cases treated with streptomycin, temperature became normal on an average in 50 hours, with sulphadiazine in 85 hours and with sulphamerazine in 89 hours.

No serious toxic symptoms were noticed with any of the drugs.

The Manchar trial was made possible by the generous gifts of streptomycin by the Medical Research Council of Great Britain and from Messrs. Abbott Laboratories, North Chicago.

The sudden outbreak of plague in Calcutta in April 1948 necessitated the sending of a treatment unit there to carry out treatment of plague cases. As no senior officer was available to supervise a controlled trial, the unit was sent primarily to create confidence in public mind regarding availability of effective treatment rather than to test the value of any drug. The trial lasted from 1st May, 1948 to 19th June, 1948 and during this period 44 cases were admitted for treatment. The trial was conducted in the Campbell Hospital, Calcutta. Although cases were treated, as far as possible, alternately with streptomycin and sulphadiazine, all cases which were considered to be serious at the time of admission were treated with streptomycin.

DOSAGE.

Streptomycin.—This opportunity was utilized to see if streptomycin could be given every 6 hours instead of every 4 hours as in the Manchar trial. Thus, in mild cases, 0·5 g. of the drug was given intramuscularly every 6 hours and in severe cases, 1·0 g. was given every 6 hours. The daily dosage thus remained about the same as in the Manchar trial.

In a few cases in which the estimation was done, the concentration was found to be over 10 units after 6 hours and over 20 units after 24 hours per ml. of serum.

Sulphadiazine.—The same dosage as given in the Manchar trial was administered.

Results.—Of the 44 cases admitted to the hospital, 34 cases subsequently turned out to be cases of plague. Six of these have been left out of consideration, 2 being convalescent cases and 4 moribund. Results in the remaining 28 cases are summarized in the following tables :—

All cases.

Treatment.	Number of cases.	Number of deaths.	Case mortality, per cent.
Streptomycin ...	16	1	6·25
Sulphadiazine ...	12	0	0·0

Results in septicæmic cases are given below separately :—

Treatment.	Number of cases.	Number of deaths.	Case mortality, per cent.
Streptomycin ...	5	1	20·0
Sulphadiazine ...	1	1	0·0

The number of cases treated are too few to draw any conclusions.

2. Plague enquiry at Coonoor in the Nilgiris district under the Director of Public Health, Madras.

Plague enquiry in the Nilgiris district was continued during the year along with the provincial scheme for the eradication of plague in the area. The materials collected during the special plague control operations were also utilized for the purpose of this enquiry.

General.—Plague is endemic in the Nilgiris district. The special features of the district are the high average altitude, temperate climate prevailing throughout the year, various types of rodents and fleas, peculiar housing conditions, extensive cultivation of potatoes in close proximity to dwelling houses and large areas of tea, coffee and rubber plantations. The object of the enquiry is firstly to determine the various factors connected with the endemicity of plague and secondly to evolve the most effective and economic methods of plague control.

Incidence of plague.—Thirty-five villages had rodent plague of which only 16 villages reported human cases, while there was no human infection in the other 19 villages. On the whole, 33 cases of plague with 6 deaths were recorded as against 55 cases and 13 deaths during the corresponding period of the previous year.

Plague control: (1) *Cyanogas fumigation.*—The villages were grouped under five categories as follows:—

(a) Villages with no history of previous plague.

(b) Villages which were infected with plague sometime or other in previous years.

(c) Villages which were threatened with plague.

(d) Villages in which plague infection in rodents or man have been reported, and

(e) Villages with a history of repeated invasions of plague or which were infected in the previous plague season.

As regards the villages coming under group (a) no action was taken except to keep a close watch for any undue mortality amongst rats or man. In villages included in group (b), cyanogas fumigation of rat burrows and other rat-harbourages was carried out as a routine once in every three months. With regard to villages coming under group (c), immediate fumigation of rat-burrows and rat-harbourages were instituted, irrespective of the fact whether the usual quarterly fumigations were already carried out or not. In villages coming under group (d) block fumigation was arranged immediately after a rat fall or the occurrence of human cases or just at the commencement of the plague season. In block fumigation all the houses included in the block are fumigated systematically and thoroughly.

Villages coming under group (e) were thoroughly fumigated, i.e. all the houses were fumigated completely, block by block, and the fumigation was repeated at monthly intervals till the plague season was over. The plague season in the Nilgiris usually commences in June and continues till about the end of February. The prevalence of plague is, however, not usually severe during the period August to October. The period March to May is comparatively free. As a result of this intensive cyanogas fumigation work, only 16 villages reported human cases of plague this year with a total of 33 attacks and 6 deaths as against 39 villages with 55 attacks and 13 deaths in the corresponding period of the previous year. The municipal towns of Ootacamund and Coonoor also continued to be free from plague. The steady decline in the incidence of plague has been maintained.

(2) *DDT as a pulicide.*—The results of the experimental use of 10 per cent DDT powder in plague control work in two villages have already been reported. Observations on the flea indices in these two villages were continued during the present year. Periodical rat-flea survey was done and the result showed a distinct reduction in flea-index in the DDT-treated village as compared with the control village. The flea index which was on an average of 3 and above was reduced to below 1 in the DDT-village and this reduction was maintained for a period of nearly one year. There can be no doubt that DDT has considerably reduced the rat-flea population. This observation has been confirmed in another group of villages which have been subsequently treated with DDT powder.

The use of DDT as a measure of prevention of plague has, therefore, been extended to about 60 village units. Unlike fumigation with hydrocyanic acid gas, DDT dusting was done only once in each village. It is proposed to observe the result of this measure on the incidence of plague during this plague season. None of the 60 villages which have been treated with DDT has so far shown any signs of plague infection.

(3) *Rodents and their fleas.*—Rodents obtained during the fumigation operations and those specially got from the fields were examined and classified. So also the fleas obtained from them. The chief rodents concerned with the spread of plague in the Nilgiris are the house rats (*R. rattus*), bandicoots (*B. malabarica*), mole rats (*Gunomys kok*), house mice and field mice. The percentage of fleas on these rodents do not vary materially from the figures already reported last year.

(4) *Sylvatic plague.*—Wild rodents numbering 278 were caught and 309 fleas were collected from them. The most predominant field rodent is the *Gunomys kok* which burrows very deeply into the ground. They are found in very large numbers in potato fields and compounds of dwelling houses. These rats migrate into houses when the potato season is over. Their fleas are mostly *Stivalius*. A few *Coratophyllus*, *Wilagrinus*, *Leptopsilla*, *X. cheopis*, *X. brasilionsis* and *Pulex irritans* were also found on them. The crushed spleen pulp of the rodents were rubbed into susceptible rats but none of them were infected with plague. In the same way their fleas were also ground up and injected into susceptible rats. The fleas were also found negative to plague.

REPORT OF THE MEETING OF THE PLAGUE ADVISORY COMMITTEE.

Held on the 1st December, 1948 in the Senate House, Madras.

MEMBERS PRESENT.

Major-General Sir Sahib Singh Sokhey. (*Chairman*).
Dr. S. C. Ghosal.
Lieut.-Colonel S. D. S. Greval.
Dr. V. R. Khanolkar.
Dr. R. B. Lal.
Dr. C. G. Pandit.
Dr. K. V. Venkatraman.
Dr. P. M. Wagle. (*Secretary*).

The following also attended :—

Lieut.-Colonel M. L. Ahuja.
Dr. A. C. Banerjea.
Lieut.-Colonel Barkat Narain.
Dr. E. Benjamin.
Major-General A. C. Chatterji.
Dr. B. C. Das Gupta.
Dr. B. B. Dikshit.
Dr. P. M. Francis.
Dr. P. V. Karamchandani.
Lieut.-Colonel C. K. Lakshmanan.
Dr. R. M. Mathew.
Dr. A. R. Mehta.
Dr. B. Mukerji.
Dr. Ananthaswmay Pillay.
Lieut.-Colonel T. C. Puri.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. D. N. Roy.
Dr. N. K. Tenaja.
Dr. N. Veeraraghavan.
Dr. K. Vishwanathan.

I. *Investigation of epidemiological factors underlying plague epidemics in Calcutta.*

A proposal for the grant of Rs. 64,500 for the year 1949-50 for investigation of epidemiological factors underlying the plague epidemic in Calcutta which was submitted by Dr. R. B. Lal was considered first. The Chairman said that, as the investigation of the factors concerned in the epidemiology of plague would require a long-time study, an organization of a permanent or semi-permanent nature was necessary. Dr. Lal explained that the investigation aimed at the elucidation of the various factors during epidemic and inter-epidemic periods.

General A. C. Chatterji, Director of Health Services, West Bengal, was in favour of a permanent organization and thought it would be possible to make provision for this enquiry, in view of its importance, from the sum of Rs. 9,00,000 asked for from the West Bengal Government for 1949-50 for control measures against plague. Dr. Raja, Director-General of Health Services, favoured the organization to be put on a permanent basis and suggested that it would be

desirable if most of the bacteriological work connected with the plague strains in this investigation was done in the Haffkine Institute, Bombay, while that which had to be done on the spot was done in the All-India Institute of Hygine & Public Health, Calcutta. The question arose about the cost of equipment needed. Dr. Lal particularly asked for a bulk sterilizer. Colonel Lakshmanan agreed to ask the Government of India for a provision for a sterilizer.

The Committee agreed that the best way to carry out the enquiry proposed by Dr. Lal was to request the Government of West Bengal to sanction a suitable organization to take up the work on a long term basis, and that any equipment needed should be provided by the Government of India by sanctioning an additional grant to the All-India Institute of Hygiene & Public Health, Calcutta. General Chatterji told the Committee that he appreciated the importance of this enquiry and assured the Committee that he would do everything possible to get the Government of West Bengal to sanction the necessary funds.

At this stage it transpired that the enquiry was already underway and the question arose of meeting the expenditure of the investigation from November 1948 to February 1949 (inclusive). The Chairman asked Dr. Lal and General Chatterji to estimate the sum required. Dr. Lal prepared an estimate which showed that a sum of Rs. 16,392 will be needed till the end of the present financial year.

Dr. Khanolkar wanted to know how the expenses of the enquiry, started some time ago, had been met and pointed out that it would be against the rules of the Association for the Executive Committee to sanction any funds without the concurrence of the Scientific Advisory Board.

Dr. Pandit, Secretary, Indian Research Fund Association, explained that the Executive Committee had not sanctioned any funds and that it will await the recommendation of the Scientific Advisory Board.

The Chairman then explained the items of the expenditure amounting to Rs. 16,392 asked for by Dr. Lal for the enquiry up to the end of the present financial year. General Chatterji explained that the budget grant that he has asked for from his Government would be available from 1st November and he believed he would find it possible to provide the sum of Rs. 16,392 from this grant. In case, however, he did not succeed in getting this grant, he wanted the Indian Research Fund Association to provide this sum.

The Committee passed a resolution asking the Scientific Advisory Board to sanction this sum. The Committee thanked General Chatterji for the very valuable assistance he had offered and assured him that plague control measures which the Bengal Government was planning would gain a great deal by being conducted on a research basis.

II (i) *Plague researches under the Director, Haffkine Institute, Bombay.*

Sir Sahib Singh Sokhey then explained the improvement brought about in the plague vaccine by the addition of tween 80 to the casein hydrolysate medium, which produced a more abundant, less clumpy and more uniform growth of the plague bacillus. He also mentioned about the raising of the incubation temperature to 32°C. in order to make the vaccine free from clumps. Dr. Venkatraman inquired if the count of the organisms had been done to which

Sir Sahib Singh replied that the count had gone up from 400-500 million to 1,000 million organisms per c.c. in a 36-hour period of incubation as a result of the addition of tween to the medium.

Dr. Wagle described the experiments carried out to determine the anti-biotic action of polymixin in experimental plague infection in white mice. These experiments were carried out at the request of Dr. Long, Director of John Hopkins' School of Public Health. The experiments showed that polymixin had a weak action against the plague organism, but was too toxic to permit of any adequate quantities being administered to decide the issue.

He then described at length the clinical field trials carried out to determine the therapeutic value of streptomycin in human plague infection. The action of streptomycin was contrasted with the action of sulphadiazine and sulphamerazine. In the trial 251 cases in all were treated in strict rotation. Of these cases 56 had septicaemia when the treatment was commenced. Of these 32 were treated with streptomycin and 3 died. Of the 11 cases treated with sulphadiazine 2 died and of the 13 cases treated with sulphamerazine 5 died. The percentage mortality was lowest with streptomycin (9·3 per cent). Besides lowered mortality rate, it was noticed that streptomycin brought down the temperature much more rapidly than the other drugs—clinically the drug seemed to be much superior to other drugs.

Dr. Karunachandani said that in cases treated in Madras Province with streptomycin he had also found streptomycin to be superior to sulpha drugs and that the temperature had reached normal earlier in cases treated with streptomycin. On Sir Sahib Singh emphasizing the importance of field trials in testing new drugs in the field, the Committee recommended the continuation of plague researches under the Director, Haffkine Institute, Bombay and recommended to the Scientific Advisory Board to approve the grant of Rs. 25,417 for the continuation of the enquiry.

II (ii) *Plague enquiry at Coonoor in the Nilgiris under the Director of Public Health, Madras.*

Dr. Francis then described the work done in the Nilgiris. He explained how DDT was found to have reduced the flea-index in the village treated by it and maintained it so for a year with the single application of DDT. The use of DDT had since been extended to 60 villages and none of the villages so treated had upto the time of report shown any signs of plague. He had continued his observations on sylvatic plague and even in this area had found no evidence of the existence of sylvatic plague.

Dr. Khanolkar wanted to know more especially the observations made and the results obtained.

Dr. Venkatraman supported the continuation of the enquiry but suggested that the points raised by Dr. Khanolkar should be borne in mind. Dr. Pandit said that he knew that a considerable amount of work was done by the enquiry during the last five years and that a report of this work was already submitted by Dr. George last year in which findings on flea survey and sylvatic plague in the Cumbum valley and North Arcot district were given. There was still much material which had remained to be sifted and correlated, and it was necessary for someone to sit down and do it. He and Dr. Mathew said that the Madras Government were spending about three lakhs of rupees on plague-control

measures, and the grant asked for was merely a token grant. They were in favour of continuation of the enquiry as that provided an opportunity for the workers to come in contact with other workers of the Indian Research Fund Association and discuss the problem with them.

The Chairman drew attention to the valuable data produced by the enquiry pointing to the fact that sylvatic plague did not exist in these areas. Dr. Mathew agreed to delete the amount asked for the purchase of DDT and said he would purchase it from the grant of his Department.

The Committee agreed to recommend to the Scientific Advisory Board the reduced budget of Rs. 5,050 in place of original sum of Rs. 7,496 asked for.

III. *Consideration of the note prepared by Dr. P. M. Wagle on the use of new insecticides in the control of plague.*

Lastly, the Committee considered the note on the use of new insecticides in the control of plague. Dr. Vishwanathan stated that some more work had since been done in the Bombay Presidency with DDT and from this work he felt that intensive spraying of floors and walls up to 4 feet of houses with DDT emulsion in infected villages was more likely to bring about subsidence of plague infection than mere insufflation of rat-burrows. Dr. Mehta inquired of the effect of this on flea-index to which Dr. Vishwanathan replied that spraying of floors and walls of the houses with DDT emulsion had brought about a very considerable reduction of the flea-index.

Dr. Wagle pointed out that the data presented by Dr. Vishwanathan showed that the insufflation of rat-burrows or the spraying of the floors and walls alone had reduced the flea-index as well as the incidence of plague infection. Recurrence of plague had, however, also occurred in villages treated in these two different ways. He contrasted this fact with the work reported from the Nilgiris where treatment of rat-burrows etc. of villages with DDT prevented any plague epidemic from occurring at all.

Sir Sahib Singh commented that it was necessary to do more work on the subject before any definite conclusions could be drawn and recommended that such work on a large scale should be carried out in all provinces.

CLINICAL RESEARCH.

1. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

Studies on the experimental production of liver cancer in rats by feeding p-di-methyl-amino-azo-benzene.

The study on the experimental production of liver cancer in rats by feeding p-DAB, which was in progress last year has been completed. Twenty-four groups of rats belonging to Haffkine and Wistar strain were used for the experiment. Each group consisted of four males and four females. The diet for the experimental rats consisted of unpolished rice mixed with p-DAB at 0·06 per cent level and was supplemented by carrot and lettuce, while the control rats received the same diet without the addition of the carcinogen. Rats on laboratory stock diet were also used as further controls. Biochemical and histological studies on livers of these rats were carried out for 9 months by sacrificing one group at the end of every month up to six months and the last group at nine months. Animals belonging to control groups were sacrificed at the commencement of the experiment, and after 3 and 6 months. The findings regarding precancerous lesions and tumour incidence are summarized in the Table :—

Table.

Duration of the experiment (months).	Duration of carcinogen administration (months).	Number of rats :		Sex.	Number of rats with precancerous or cirrhotic changes in the liver.		Number of rats with liver cancer.	
		Haffkine.	Wistar.		Haffkine.	Wistar.	Haffkine.	Wistar.
0	0	4	4	M	...	...	...	...
		4	4	F	...	...	...	...
1	1	4	4	M	...	...	...	...
		4	4	F	...	...	...	...
2	2	4	4	M	...	2	...	...
		4	4	F	...	2	...	1
3	3	4	4	M	...	...	...	...
		4	4	F	...	2	...	...
3	0	4	4	M	...	...	...	...
		4	4	F	...	...	...	...
4	4	4	4	M	...	2	...	...
		4	4	F	...	...	...	3
5	5	4	4	M	...	4	...	...
		4	4	F	1	4	...	1
6	6	4	4	M	2	4	1	1
		4	4	F	2	4	2	2
6	0	4	4	M	...	...	...	...
		4	4	F	...	...	...	...
9	9	4	3	M	2	3	1	1
		4	5	F	...	1	...	4

Biochemical studies.

Quantitative estimations of cholesterol, phospholipids, ATP system, sulfhydryl compounds and respiratory enzymes in liver tissues of the experimental and control rats were carried out.

Cholesterol.—No appreciable difference was observed in the cholesterol content of the livers of control rats sacrificed at the commencement of the experiment and at 3 and 6 months. The carcinogen treated rats showed fluctuations in liver cholesterol values, which were more pronounced for the females of the Wistar strain.

Phospholipids.—The organic phosphorus is an index of the phospholipids in the liver. It showed the following alterations when considered in relation to body-weight and liver-weight of the animal. The level of organic phosphorus for the control rats on stock diet and on rice diet remained unaltered throughout the experiment. In the case of experimental rats on p-DAB diet the liver organic phosphorus increased 2¼ times its value in Haffkine rats although there was no appreciable change in Wistar strain rats. The significance of these changes is difficult to evaluate at present, except to state that the carcinogen upsets the lipid storage and metabolism in the liver.

ATP system.—The adenosine triphosphate and phosphocreatine contents of livers were studied with a view to detecting any alterations in phosphate metabolism. There appears to be an abrupt rise about the fifth month in respect of both these constituents but the results are offset to a certain extent by a corresponding high value in Haffkine rats on control diet at about six months. No significant alterations were found in inorganic phosphate and adenosine triphosphatase during the whole of the experiment.

Sulfhydryl compounds.—A study of sulfhydryl compounds in the liver tissue was attempted by estimating glutathione, cystine and methionine. No significant difference could be detected in the quantities of these constituents at any age group between the two strains and between the control and the experimental animals. This appears to be a very unexpected finding as the involvement of the sulphydryl group in the growth process and, therefore, in cancer has been emphasized by Hammett and co-workers and also suggested by the experiments of Crabtree.

Succinoxidase (respiratory enzyme).—There is at first a moderate rise for the first two months in the succinoxidase activity as evaluated by succinate or p-phenylene-di-amine effect. This is followed by a steep fall between the second and third month with a further gradual decline until the sixth or seventh month when the cancer appears in the liver. This is a very striking finding that was suggested by the work of Salter and others in experiments which had not been so vigorously designed.

Investigations on cancer patients.

The work carried out during the year was a continuation of the work described in the last report. The studies have been extended to patients suffering from cancer of cervix uteri. It is not possible to give the results at present, as the work is still in progress.

2. Naga sore enquiry under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong.

The enquiry was considerably handicapped owing to the very low incidence this year of Naga sore in Assam and the difficulty in securing cases for investigation. The transfer of Dr. M. N. Lahiri, Research Officer, towards the commencement of the Naga sore season, was also a loss to the enquiry.

During the period under report, only one case was admitted with a sore on the dorsum of the foot of seven weeks duration, resulting from injury from an axe. The sore was in the stage of healing. *Fusiformis* were present in the discharge but were not constant in day-to-day smears. No *spirochaete* was present. The *fusiform* bacillus could not be cultivated either in Wright's medium or the usually employed digest agar, both enriched with 20-per cent sheep blood. The patient was somewhat undernourished and had microcytic anæmia but had no blood or intestinal infection.

A second case which refused admission had a circular ulcer about an inch in diameter with a profuse mucoid discharge, situated on the ventral aspect of the leg over the calf muscles and resulting from an injury. Smears teemed in *fusiformis* and *spirochætes* as if almost in pure culture. Growth of the former resulted in primary cultures on blood-agar plates but the organism failed to grow in subcultures. The patient was not available for a repetition of the cultures nor for a full investigation of his general condition. He was well nourished.

At the time of writing, arrangements have been made for despatch of three cases from a tribal area in Assam's frontier.

In contrast to the earlier strains which were stated to be non-motile, the two strains of *fusiformis* isolated late last year were found to be motile. The motility is brisk in 24-and 48-hour cultures and even at 72 hours motility can be made out, but thereafter when growth is at its peak, it is practically non-existent except perhaps for an occasional sluggishly moving bacillus. The earlier strains having died owing to an accident, they were not available for re-examination, but being morphologically and culturally identical with these two recent strains, one may not be wrong in assuming that they too were motile, the motility having been missed because of their examination at a later stage of growth.

In regard to the other cultural and biochemical reactions, these two recent strains were similar to the earlier strains. In sugar media enriched with 15 to 20 per cent sheep serum, growth precipitating to the bottom of the tube (saline agglutinable growth) resulted, but there was no acid formation. A small quantity of gas was, however, generated in glucose and maltose but not in lactose, saccharose, dulcite and mannite. In sugars enriched with defibrinated sheep blood the results were the same except for slight acid formation in glucose and maltose. There was no gelatine liquefaction even in blood-enriched medium; indole was produced. The organism is highly pleomorphic, very lengthy filaments, beaded forms etc. being seen in older cultures. The organism grows in cooked meat medium with or without added blood incubated ærobically. In glucose Douglas agar deeps, ærobically, there is a growth along the line of the stab with slight side-outgrowths, commencing from a point about 2 cm. from the surface.

3. Enquiry on the rôle of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

Introduction.

The incidence of portal cirrhosis of the liver in India is fairly high. It is a chronic disease, usually fatal and commonly seen in the poorer classes of the people. The causative factors in this disease appear to be multiple. The onset is usually insidious and the disease runs a chronic course. Preliminary investigations regarding the ætiology of the condition have shown that alcohol appears to have a very insignificant rôle and that dietetic deficiencies appear to be important factors in the causation of the disease (Radhakrishna Rao, 1936, 1937).

In contrast to the portal cirrhosis of adults a progressive and rapidly fatal cirrhosis commonly known as 'infantile biliary cirrhosis' is prevalent in Indian children belonging to middle and rich class families. This disease appears peculiar to India and takes a high toll of life among Indian children. The ætiology of this disease is still obscure.

The question being of such immense importance, it appeared that a study of the nutritional factors responsible for liver damage might materially help towards devising ways and means to prevent or modify such changes in the liver.

The problem was undertaken in its experimental and clinical aspects. The experimental work consisted in producing hepatic damage with various deficient diets and studying the effect of vitamins of the B_2 complex, choline and methionine. The clinical investigations were mainly concerned with studies on the effect of treatment of decompensated portal cirrhosis with high-protein, high-caloric diets with vitamin supplements.

(A) *Clinical investigations in cirrhosis of liver.*

Object.—

(a) To study the changes in serum protein fractions in cirrhosis of liver.

(b) To determine whether the choline concentration in the blood is of any importance in the clinical picture of cirrhosis.

(c) To study the effect of treatment with high caloric, high protein diets with vitamin supplements.

Conclusions already drawn.—

(a) A common finding is the fall in the albumin and rise in globulin fraction. The albumin : globulin ratio shows a reversal from the normal value. In the globulin fraction the euglobulin shows the highest percentage increase and the course of the disease is reflected in the albumin and euglobulin changes.

(b) In many cases of portal cirrhosis the serum-choline values do not show any appreciable difference from the normal.

(c) A high protein diet which includes casein hydrolysate and skimmed milk, with vitamin supplements or liver extract, has a definite ameliorative effect on the clinical condition of the patient and the progress of the disease.

The dietary treatment of adult and juvenile patients with cirrhosis of the liver by high protein vitamin supplemented diets are well in progress. Casein hydrolysate and skimmed milk for this study were supplied from this Institute.

Dr. N. J. Modi, M.B.B.S., M.R.C.P.(Lond.), Honorary Physician, Goculdas Tejpal Hospital, Bombay, collaborated with us in the adult cirrhosis study. The investigations on juvenile cirrhosis were made possible by the assistance of Dr. R. V. Sanzgiri, M.R.C.P.(Lond.), Honorary Physician, Bai Jerbai Wadia Children's Hospital, Bombay.

The normal data given here are mostly obtained from the staff of the Institute.

Results.—The changes in the serum protein fractions as determined by Howe's method of 32 adult cirrhosis patients and 20 juvenile cases have been studied. For comparison other diseases like anæmia, malaria, cardiac failure, etc., were also studied.

Subjects.	Number of cases.	Total protein g./100 c.c. serum.	Albumin g./100 c.c. serum.	Globulin g./100 c.c. serum.	Euglobulin g./100 c.c. serum.	Pseudo-globulin g./100 c.c. serum.
Normal subjects	22	6·90	4·57	2·33	0·46	1·87
Adult cirrhosis	32	6·12	2·34	3·78	1·36	2·42
Juvenile cirrhosis	20	6·43	2·80	3·63	0·92	2·71
Other diseases	16	5·57	2·26	3·31	1·38	1·93

The decrease in albumin and increase in globulin without any appreciable difference in the total protein content in cirrhosis cases is evident. In other diseases, the lowering in albumin is accompanied by slight decrease in total protein level also. The level of euglobulin gives an idea as to the progress of the disease.

In addition to protein fraction studies, choline estimations were made in normal and cirrhosis cases with a view to find out whether their concentrations in blood have any clinical significance.

The results are given below :—

Subjects.	Number of cases.	Choline in mg. per 100 c.c. of serum.
Normal subjects ...	17	20·8
Adult cirrhosis ...	10	16·7
Infantile cirrhosis ...	9	18·6

The nitrogen excretion in urine was also studied in a few cases of cirrhosis. For the sake of comparison, analysis of normal urine was also done. The patients were on a high-protein diet of eggs, skimmed milk and casein hydrolysate. Plasma and whole-blood transfusion were given in many cases. The excretion of total nitrogen in 24 hours' urine was in all cases lower than normal. This shows that the protein supplied is utilized in the body though this was not evident from the blood-protein values.

The ratio of amino nitrogen in total nitrogen excreted was found to be always greater in cirrhosis cases than normal. This could not be attributed to the administration of casein hydrolysate, as even before treatment this ratio was higher than normal.

(B) *Experimental work.*

The biochemical and histological changes in the liver were studied, resulting from :—

1. Continued deprivation of factors present in vitamin-B_2 complex.
2. Varying levels of dietary protein intake.
3. Restricted intake of protein in an otherwise adequate diet.
4. High-fat low-protein and high-carbohydrate low-protein diets supplemented with synthetic vitamins of the B_2 group (excluding choline).
5. Same as (4) but supplemented with choline and methionine.
6. High-fat and high-carbohydrate diets with yeast protein instead of casein with synthetic vitamins.

The details of the experiments are given in the Protocol (*see* p. 111).

Methods.—Young healthy albino rats were equally distributed in different groups with respect to weight, sex and litter mates. Animals used in the first two experiments had initial weights varying between 40 g. and 50 g. while for the other experiments the initial weight was generally more than 90 g. The rats were housed individually in single cages and food and water supplied *ad libitum* unless otherwise stated. The animals were weighed weekly and a record of their food consumption was also maintained.

The casein was purified by dissolving in a solution of weak alkali and precipitation with dilute acid. The precipitated casein was washed free from acid and dried in the sun. The starch was purified by treating it with dilute solution of alkali. 0·5 g. of Squibb's Brewer's yeast was given to animals of the control group and other experiments to supply the vitamins of B group. In the 16-per cent yeast diets Brewer's yeast was used.

Except in the restricted protein intake group, where only measured quantity of food was given, the animals were given food and water *ad libitum* in all other experiments. From the amount of food left over the food consumption was calculated. Vitamin supplements, choline and methionine were given in the form of pills which the animals consumed readily. Osborne and Mendals salt mixture No. 185 was used.

Histological technique.—The animals were killed by ether anæsthesia and their livers were dissected and weighed immediately. A suitable portion of the liver was taken for histological examination and the remaining portion was used for chemical examination. For histological examination, sections of liver and kidneys were prepared by staining with Ehrlich's hæmatoxylin and eosin. For collagen fibres Weigert's iron hæmatoxylin and van Gieson's stain was used. The silver impregnation method of Gomori was used for staining reticulum.

Chemical estimations.—Moisture, fat and protein content of the tissues were determined by the usual methods. The choline content of the liver was estimated by the method of Engal (1942) by precipitation as choline reineckate, while in blood the micro-method of Erickson *et al.* (1940) was followed. Blood proteins were determined according to Howe's procedure (1921).

Results.—

Experiment 1 : This experiment was designed to permit observations on the possbile inter-relationships between vitamin-B_2 complex and protein as they affect the liver. The average results are given below :—

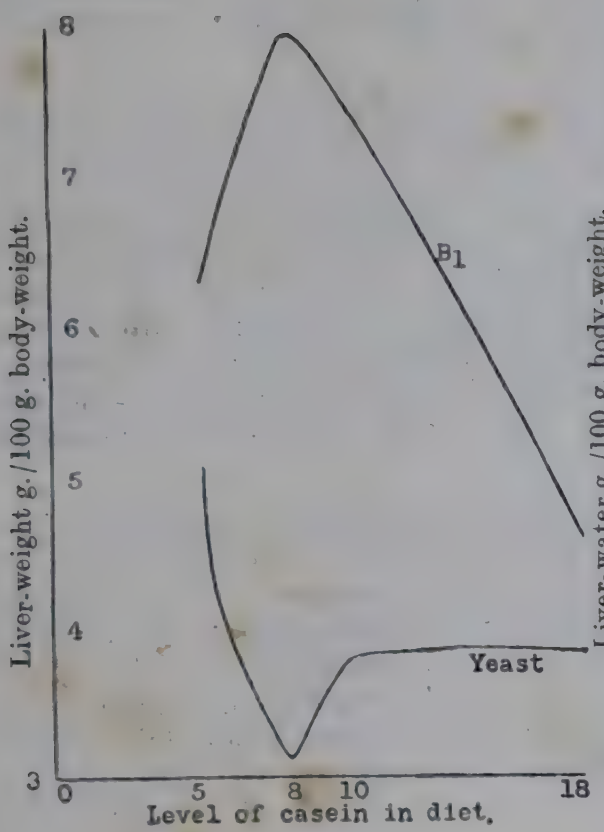
Diet.	Initial weight in g.	Final weight in g.	Liver weight g./100 g. body-weight.	Liver water g./100 g. body-weight.	Liver fat g./100 g. body-weight.	Liver protein g./100 g. body-weight.
(a) 18-per cent casein *plus* B_1 ...	41·8	105·4	4·54	3·10	0·47	0·87
(b) 10-per cent casein *plus* B_1 ...	38·8	73·3	8·45	5·32	1·45	1·30
(c) 8-per cent casein *plus* B_1 ...	35·4	44·2	8·00	5·15	1·16	1·25
(d) 5-per cent casein *plus* B_1 ...	38·4	27·3	6·31	4·05	1·15	0·94
(e) 18-per cent casein *plus* yeast ...	46·0	187·3	3·85	2·75	0·17	0·79
(f) 10-per cent casein *plus* yeast	42·4	194·0	3·82	2·81	0·19	0·73
(g) 8-per cent casein *plus* yeast	43·0	194·2	3·10	2·21	0·14	0·61
(h) 5-per cent casein *plus* yeast ...	43·7	56·3	5·09	3·72	0·36	0·77

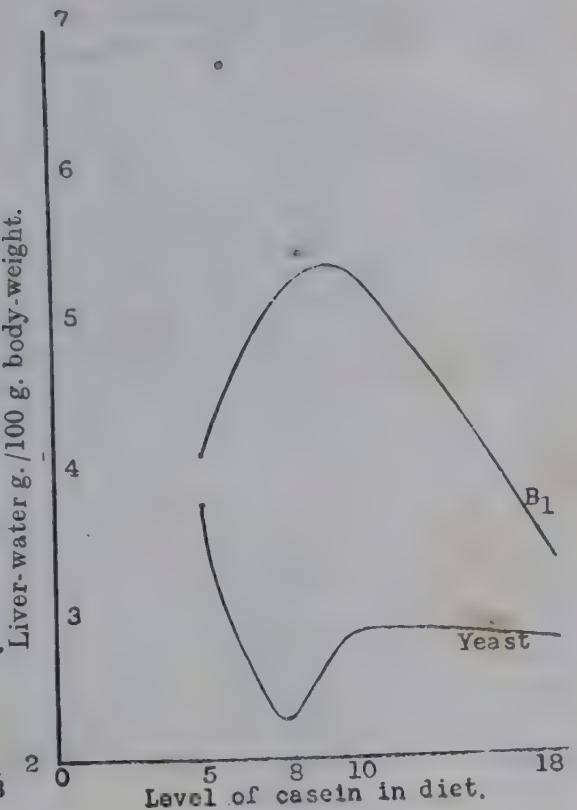
It is customary to express the results of liver analysis as percentage of fresh liver-weight but it has now been generally agreed that a clearer idea of the changes in any organ in relation to total body-metabolism is obtained when the absolute amounts of the different constituents are expressed as g./100 g. body-weight.

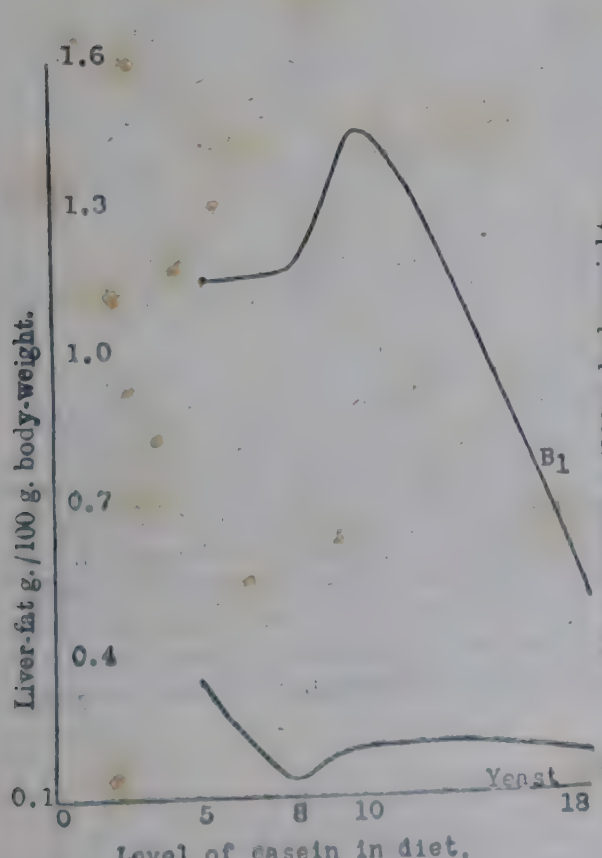
The Graphs clearly show that at some optimum level of dietary protein, between 5 and 18 per cent the deficiency of vitamin B_2 is most prominent as shown by liver-weight, fat and water content.

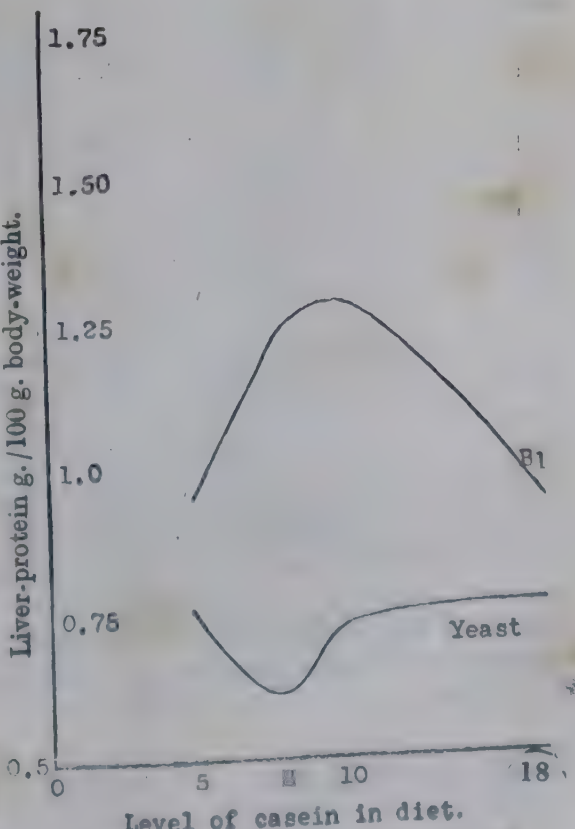
The B_2 complex deficient rats were characterized by higher mortality and shorter period of survival as compared to the controls. The increase in their weights was proportional to the amount of casein in the diet. The B_2 deficiency is less acutely felt in the low- and high-protein groups than in the two groups in-between. Though the individual values of the fat and protein contents of the liver have no direct bearing on the level of protein intake, the ratio of fat to protein usually increases with the decrease in dietary protein in both the B_1 and yeast groups. Another distinct feature is the constant value for liver protein in the yeast group, and the remarkably narrow range of variation in the protein values even in the B_2 deficient group.

Histological findings.—Livers of rats given yeast as supplement showed that the liver parenchyma retained its normal histological structure in the groups receiving 18-per cent and 10-per cent protein. The rats in the 8-per cent and 5-per cent protein groups killed late in the experimental period showed


Liver-weight g./100 g. body-weight.
8
7
6
5
4
3
B1
Yeast
0
5
8
10
18
Level of casein in diet.


Liver-water g./100 g. body-weight.
7
6
5
4
3
2
B1
Yeast
0
5
8
10
18
Level of casein in diet.


Liver-fat g./100 g. body-weight.
1.6
1.3
1.0
0.7
0.4
0.1
B1
Yeast
0
5
8
10
18
Level of casein in diet.


Liver-protein g./100 g. body-weight.
1.75
1.50
1.25
1.0
0.75
0.5
B1
Yeast
0
5
10
18
Level of casein in diet.

slight fatty infiltration around the central veins. In both the groups there was a rarefication in the cytoplasm which may be due to an excess deposition of glycogen (Best *et al.*, 1948; Franklin *et al.*, 1948).

In the 18 per cent and 10-per cent protein groups receiving only vitamin B_1 as supplement, the liver parenchyma showed moderate to serve fatty infiltration, particularly around the central veins (even as early as 70 days). Traces of ceroid pigment was also visible in these areas. In the 8-per cent and 5-per cent protein groups extensive fatty infiltration, more marked around the central veins, was seen. An interesting feature in the 8-per cent protein group was the appearance of replacement fibrosis, patchy in distribution, around the central veins. In certain cases this was more marked and there was a tendency for the formation of pseudolobules. The 5-per cent protein group did not show any marked replacement fibrosis. None of the animals showed massive necrosis.

Experiment 2: An interesting feature of the previous experiment was the absence of massive necrosis of the liver in any of the rats receiving either low-protein B_2-deficient diet or low protein yeast supplemented diet. This is in contrast to the results obtained by Himsworth and Glynn (1944) who claimed to have produced massive hepatic necrosis in rats by feeding low-protein diets in which the daily dietary intake of casein was between 200 mg. and 500 mg. Therefore, it was thought worthwhile to study the histological and biochemical changes in the liver of rats on a restricted intake of protein. The average results are given below :—

Daily casein intake g.	Initial weight in g.	Final weight in g.	Calories intake.	Liver Weight g./100 g. body-weight.	Water g./100 g. body-weight.	Fat g./100 g. body-weight.	Protein g./100 g. body-weight.	Ash g./100 g. body-weight.
(a) 1·2	37·8	170·3	32·66	3·24	2·28	0·17	0·65	0·05
(b) 0·6	37·5	149·5	28·6	2·87	2·04	0·17	0·55	0·04
(c) 0·4	36·0	139·0	26·7	3·16	2·19	0·31	0·51	0·04
(d) 0·2	38·8	89·0	19·3	4·16	2·81	0·48	0·65	0·05

The results show that the growth-rate and caloric-intake of the different groups of rats parallel the daily casein-intake. Though the increased protein-intake has no appreciable effect on the protein-content of the liver, its effect on the fat-content is evident. Only the 0·2 g. group showed any considerable variation in liver weight, liver water, and liver-fat. Otherwise the findings in the rest of the groups are more or less the same. The uniform changes in the total plasma-protein concentration of the rats of the different groups provided the best evidence of the uniform daily intake of protein in the same group of rats and the different levels of casein-intake in the different groups of rats. The values of total protein and choline in mg./g. of fresh-liver tissue are given below .—

Protein (casein) intake ...	1·2 g.	0·6 g.	0·4 g.	0·2 g.
Plasma-protein g./100 c.c. plasma	6·83 g.	6·11 g.	5·52 g.	4·81 g.
Choline mg./g. of fresh liver tissue	2·14 mg.	1·89 mg.	1·43 mg.	1·55 mg.

The growth-curves of animals in these groups showed a definite downward trend as the protein-intake of each group is lowered. The animals became emaciated with low-protein intake. Except slight pallor or mottling in the low-protein groups, the liver showed no macroscopical changes.

Histology.—On microscopical examination, the liver in animals which received 1·2 g. and 0·6 g. of protein per rat per day showed no pathological changes. Animals which received 0·4 g. and 0·2 g. of protein per rat per day, on the other hand, showed slight fatty change of the hepatic parenchyma around the central veins. In marked contrast to the animals in the low protein groups which were deprived of vitamins of B_2 complex, none of the animals in this group, however, showed marked fatty change in the hepatic parenchyma around the central veins, accumulation of ceroid pigment or diffuse hepatic fibrosis.

Experiment 3 : This experiment was designed to study the effect of high-carbohydrate low-protein and high-fat low-protein diets on the liver. The average results are given below :—

Diet.	Initial weight in g.	Final weight in g.	Calories intake.	Food intake in g.	Liver weight g./100 g. body-weight.	Water g./100 g. body-weight.	Fat g./100 g. body-weight.
(a) 6-per cent protein *plus* 50-per cent fat (Dalda)	119	67·0	17·9	2·53	7·07	3·91	2·22
(b) 6-per cent protein *plus* 85-per cent carbohydrate	135	110·4	22·8	5·33	4·70	2·83	0·99

Animals fed on the high-fat diet showed a considerable decline in body-weight. A number of them developed œdema of the extremities and some ascites. Food consumption was very low, and the animals did not survive long. The animals receiving high-carbohydrate diet, on the other hand showed only a decrease in weight in the initial period but subsequently maintained a constant body-weight and lived much longer than those in the high-fat group.

The water and fat content of the liver in the high-fat group showed appreciable deviation from the normal values. The low-choline concentration of 1·06 mg./g. of fresh liver tissue suggests a reduction in the choline containing phospholipids in the liver. The total protein concentration of plasma was also low, being 3·77 g./100 c.c. plasma.

On the high-carbohydrate diet, the liver-weight and the fat-content of the liver are comparatively lower than those on the high-fat diet. The total proteins of plasma was 5·38 g. per 100 c.c. plasma the albumin concentration being 2·97 g.

Histology.—Livers on macroscopical examination of most of the animals on the high-fat diet showed varying degrees of enlargement and were highly fatty. In some instances, the organ was extremely resistant to cut. On histological examination severe fatty infiltration was found in the liver in

almost all the animals but none showed massive necrosis of the parenchyma. Animals killed between 55 and 97 days showed diffuse hepatic fibrosis and in some typical cirrhosis with ceroid pigmentation was found.

Animals of the high-carbohydrate group showed varying degrees of enlargement of the liver, which appeared fatty and granular in many cases. The consistency of the organ also varied from animal to animal and in one case it was resistant to cut. Histologically marked fatty infiltration in the liver was found in almost all the animals. About half the number killed after 150 days showed replacement fibrosis, particularly around the central veins. Typical cirrhosis with ceroid pigment was found in one instance.

Histological examination of the liver in both the above groups shows that the fatty change in the organ is not proportional to the period of experimental feeding. In general, the deposition of fat in the liver is more marked during the period of growth of the animal.

The formation of fibrous tissue in dietary hepatic cirrhosis is still a controversial subject. From a study of several sections of the liver at different stages of the development of fibrous tissue it appeared that the fibrous tissue is first formed around the central veins and, later, extended to the portal systems.

Blumberg and McCollum (1941) and others have shown that typical cirrhosis can be produced in rats fed on high-fat diets low in choline, similar to those used in this study, within a period of 100 to 150 days. Our results indicate that similar lesions can be produced in the liver in a much shorter period, namely within 100 days.

Experiment 4 : These experiments were planned to study the action of choline and methionine on the liver of rats on high-fat diets with low-protein intake. The average results are given below :—

Diet.	Initial weight in g.	Final weight in g.	Food intake in g.	Calories intake.	Liver weight g./100 g. body-weight.	Water g./100 g. body-weight.	Fat g./100 g. body-weight.
(a) 50-per cent fat *plus* vitamin supplements	102	62	2·53	16·3	6·76	4·07	2·10
(b) 50-per cent fat *plus* 4 mg. choline	98	65	2·60	17·0	5·62	3·83	0·49
(c) 50-per cent fat *plus* 20 mg. methionine	96	60	2·86	18·7	4·50	3·04	0·56
(d) 30-per cent fat *plus* vitamin supplements	98	100	4·67	25·7	7·98	...	...
(e) 30-per cent fat *plus* 8 mg. choline	97	91	4·80	26·4	5·40	...	...

These experiments demonstrate clearly that choline has no effect either on the food consumption or the growth rate. Animals fed 4-mg. choline showed striking decreae in liver-weight and water and fat-content of the liver, though at the dosage supplied, these values have not returned to normal. The choline-content of the liver of animals receiving 4-mg. choline supplement was on an average 1·18 mg./g. of liver-tissue which is not much different from the values obtained in other groups. The total plasma-protein was, however, higher in this group as compared to the control, its value being 4·93 g./100 c.c. In animals receiving a supplement of 6-mg. choline the liver was nearly normal in weight.

Histology.—

(a) The control animals fed 50-per cent fat diet showed histological appearances similar to those already reported in the previous experiment.

(b) Animals given 50-per cent fat with 4 mg. choline showed slight emaciation, but the livers were fairly normal in size. In few instances, the colour of the organ was slightly lighter than normal and showed mottling. In this group the fatty change in the liver was much less marked as compared to the controls. Similarly, the fibrosis was also less marked and there was no accumulation of ceroid pigment.

From the histological examination, it appears that a daily supplement of 4-mg. choline chloride is not sufficient to completely prevent the fatty change in the liver.

(c) Animals given 50-per cent fat with 20 mg. of methionine were also emaciated but lived longer. The liver was slightly pale in colour but was otherwise normal. In this group there was moderate fatty change in the liver with slight accumulation of ceroid pigment. Compared to the above, the fatty change in this group is more marked. Slight fibrosis around the central veins was also observed in a few animals.

(d) Animals given 30-per cent fat diet without choline were comparatively better than those on the corresponding 50-per cent fat diet. The livers were enlarged, pale and fatty. Histological examination of the liver showed typical heavy fatty infiltration, particularly around the central veins. Although ceroid pigment was occasionally noticed, no fibrosis was present.

(e) The general condition of the animals given 30-per cent fat diet with 8 mg. choline was better than those fed 50-per cent fat diet with 4 mg. choline. The livers were also normal in size. On microscopical examination fatty infiltration of the liver was not found in any of the animals of this group. There was no evidence of ceroid pigment or fibrosis in any of the animals.

Thus, the administration of 8 mg. of choline per rat per day was able to prevent fatty infiltration of the liver when the fat content of the diet was 30 per cent.

Experiment 5: In this experiment, yeast protein was used instead of casein in the production of hepatic lesions. Sixteen-per cent Brewer's yeast with two basal diets containing high fat and high carbohydrate were used. The yeast contained 45-per cent crude protein. The average results are given below:—

Diet.	Initial weight in g.	Final weight in g.	Food intake in g.	Calories intake.	Liver weight g. 100 g. body-weight.	Water g. 100g. body-weight.	Fat g. 100g body-weight.
(a) 16-per cent yeast *plus* 50-per cent fat	117	64	2·65	17·2	4·88	3·17	...
(b) 16-per cent yeast *plus* 75-per cent carbohydrate	139	97	5·18	22·1	3·47	2·52	0·18

It will be seen that these results are, in general, similar to those reported above in experiment 3. The enlargement of the liver is, however, not so marked as compared to the corresponding groups on low casein diet. The choline content of the fresh-liver tissue in high-carbohydrate diet was found to be 1·35 mg. /g.

Histology.—

(a) Animals of the high-fat group showed marked emaciation and some developed ascitis. The liver of most animals was slightly enlarged, pale and granular.

On microscopical examination extensive fatty infiltration was present in the liver of almost all the animals. Patchy fibrosis of the parenchyma was seen in nearly half the number in this group. In most cases, the fibrosis was seen around the central veins, where ceroid pigment was also present. In some instances the fibrous tissue was more marked and enclosed small lobules of the hepatic parenchyma.

(b) Animals of the high-carbohydrate group were slightly emaciated. The liver was slightly pale but normal in consistency.

Very slight fatty infiltration in the liver was seen only in three animals out of 12 killed after 137 days. There was no evidence of massive necrosis of the parenchyma of the liver in any of the animals. Similarly, the liver showed no ceroid pigment or fibrosis.

Although the experimental diets used were similar to those of Himsworth and Glynn (1944), it may be noted that massive hepatic necrosis was not evident even in a single instance.

Protocol of experiments.

Experiment number.	Total number of rats.	Number of rats in each group.	Diet: Casein, per cent.	Fat, per cent.	Carbohydrate, per cent.	Salt mixture, per cent.	Supplements per rat per day.*	Duration of the experiment, days.
1	180	(a) 24	18	8	70	4	a, b	49—476
		(b) 12	18	8	70	4	a, c	247—358
		(c) 24	10	8	78	4	a, b	34—454
		(d) 12	10	8	78	4	a, c	55—387
		(e) 24	8	8	80	4	a, b	48—306
		(f) 12	8	8	80	4	a, c	185—500
		(g) 48	5	8	83	4	a, b	7— 99
		(h) 24	5	8	83	4	a, c	7—416
2	48	(a) 12	Same as 1(a) but daily intake of casein was restricted to a maximum of 1·2 g. per rat.				a, c	149—286
		(b) 12	Same as 1(c) but the daily intake of casein was restricted to a maximum of 0·6 g. per rat.				a, c	154—290
		(c) 12	Same as 1(e) but daily intake of casein was restricted to a maximum of 0·4 g. per rat.				a, c	158—283
		(d) 12	Same as 1(g) but the daily intake of casein was restricted to a maximum of 0·2 g. per rat.				a, c	132—246
3	24	(a) 12	6	6†	85	3	d.	86—249
		(b) 12	6	51†	40	3	d.	41— 97
4	60	(a) 12	6	51†	40	3	d.	33—110
		(b) 12	6	51†	40	3	d. *plus* 4 mg. choline	33—124
		(c) 12	6	51†	40	3	d. *plus* 20 mg. methionine.	91—162
		(d) 12	6	31†	60	3	d.	42—145
		(e) 12	6	31†	60	3	d. *plus* 8 mg. choline	63—168
5	24 (experiment with yeast protein)		Yeast					
		(a) 12	16	6†	75	3	d.	83—181
		(b) 12	16	51†	30	3	d.	29—113

*a=2 drops of cod-liver oil. b=20 μg. of thiamine. c=0·5 g. of Brewer's yeast. d=20 μg. thiamine. 25 μg. riboflavine. 20 μg. pyridoxine. 100 μg. calcium pantothenate.

† Includes one per cent cod-liver oil.

4. Enquiry on cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam.

A batch of young puppies were fed on a choline-free, low-protein diet, diet I which was as follows: Protein 3·66 g. per kg. body-weight per day, carbohydrate 13·98 g. per kg. per day; fat 12 g. per kg. per day, salts 3 g. per kg. per day. All the vitamins except choline were supplemented in adequate doses. The lesions appeared after 22 days. Post-mortem examination showed extensive and diffuse fatty infiltration of the liver in 2 animals. This study was to determine direct effect of diet I in puppies.

A batch of pregnant bitches (full-term) were fed on the same diet (diet I). One of them delivered dead foetuses, another delivered 6 premature and weak puppies. These survived for a maximum period of 8 days. Another delivered 4 healthy puppies but after 9 days the puppies developed jaundice and they died one after another in 3 days. Histological examination of the livers showed some centri-lobular fibrosis with thickening round the hepatic vein and irregular areas of necrosis in the liver in the last batch. In 3 sucklings of another bitch on histological examination showed that fibrosis was very marked around the centri-lobular vein, there were collections of haemopoietic cells in these areas.

The second batch of pregnant bitches were fed on a choline-free diet with slight increase of protein and reduction of fat by 50 per cent. This diet II, consisted of protein 4·14 g. per kg. wt. per day, carbohydrate 47·52 g. per kg. wt. per day, fat 6 g. per kg. wt. per day and salts 3 g. per kg. wt. per day. All vitamins except choline were supplemented in adequate doses. The effects of this diet on the liver of the sucklings are now being studied.

Meanwhile the estimation of choline in the mother's milk of the experimental animals are determined by a microbiological method described by Horwitz (*Jour. Biol. Chem.*, 150, No. 2, p. 325). These are compared with the choline content of the milk of the normal bitches.

Similar experiments have been and are being carried out with rabbits. The same diet I was given to the pregnant rabbits and their sucklings as they grew up. After 25 days the animals lost weight markedly, lost appetite and gradually lost fur. On histological examination in the liver showed areas of necrosis with fat-loading. Experiments with diet II were also carried out. After 100 days, histological examination of the livers were done by surgical open biopsy method. Biopsies in these cases will be carried out at definite intervals to study the progress of the hepatic lesion during the experiments. These experiments have shown early cirrhotic changes with distribution of fibrosis that is suggestive.

5. Enquiry entitled "Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis" under Professors V. Subrahmanyan and V. R. Naidu at the Indian Institute of Science, Bangalore, and Sri Krishnarajendra Hospital, Mysore.

During the period under report one hundred and thirteen cases were studied and treated. Most of the cases were treated as out-patients and were under daily observation of the medical assistant who also visited them in their homes whenever necessary. A few beds had been reserved in one of the wards exclusively for cases of infantile hepatic cirrhosis. A few select cases were hospitalized for short periods for special study and also extra treatment.

In continuation of the work reported previously, the clinical investigation was confined to a study of the therapeutic action of choline chloride as compared with that of the pepsin proteolysed extract of liver and spleen supplemented with a smaller amount of choline chloride. Several liver-function tests (the thymol-turbidity, the cephalin-cholesterol flocculation and the bromosulphalein-retention tests) have been examined with particular reference to their applicability for diagnosis as well as prognosis in this disease. Certain aspects of fat metabolism and plasma constituents related to fat metabolism have been studied at different stages of the disease. Liver-biopsy studies have been continued.

The following account summarizes the results obtained and the conclusions drawn therefrom :—

1. Clinical studies—choline therapy.

The classification of the disease into the various pathological stages and the related symptomatology as also the criteria of clinical improvement and cure have all been described in detail in previous reports. Liver function tests—particularly the thymol turbidity test—and icteric index have served as confirmatory aids to clinical symptoms in the initial diagnosis and assessment of the exact pathological stage as well as for prognosis under treatment.

Eighty-seven cases at different stages of the disease were treated with choline chloride. They received pure choline chloride (Merck) in the form of a faintly acid (0·05 N HCl) 25 per cent solution of the salt in 10 per cent alcohol. The faint acidity prevents hydrolytic degradation of the salt and the alcohol protects it against bacterial contamination. The dosage was 250 mg. of choline chloride twice daily, i.e. 500 mg. per day per child which is 10 to 12 times the maintenance dose judging on the basis of data for experimental animals. The bitter salt was rendered as palatable as possible by admixture with sugar or honey prior to administration.

The rest twenty-six cases served as the control group and were treated with a pepsin-proteolysed extract of the liver and spleen supplemented with 0 mg. per c.c. of choline chloride. Each child received 2 teaspoonsful of the extract twice daily, which amounts to 140 mg. to 150 mg. of added choline chloride per child per day. The pepsin-proteolysed liver-spleen extract was prepared by enzymic hydrolysis of a mixture of liver and spleen (in the proportion 4 : 1) for 48 hours at pH 2 and at 37°C. with a fresh acid (0·08 N HCl) extract of bovine or hog gastric mucosa ; the extract was then neutralized to pH 5·5 and stored until required for use protected by the addition of alcohol to 12 per cent (V/V).

An adequate supply of vitamins was ensured in all cases by the oral administration of B-complex preparations (Neurona B-complex, etc.) supplemented with vitamin C (50 mg. to 100 mg. per day) and Adexolin.

Secondary complaints like constipation, temperature, anæmia, etc., were treated symptomatically. Bi-weekly injections of liver extract (2 U.S.P. units) and vitamins of the B-complex (B_1 5 mg., riboflavin 1 mg., nicotinic acid 25 mg., pantothenic acid 2 mg., and pyridoxine 0·5 mg.) and vitamin C 50 mg.) were given to relieve anæmia. To counteract anuria and œdema in the advanced stages of the disease diuretics were given orally and parenterally—diuretic mixture, calcium-diuretin tablets, liquid-extract of Punarnava and Kalamegh orally and acidrone and aminophylline parenterally. In most cases

there was little response to such treatment. In some cases, however, the drugs in the first instance increased the frequency of urination though the quantity voided was not markedly increased. Later, there was a relapse of severe anuria. There was no evidence of kidney damage in any of the above cases. In order to relieve icteric toxæmia in the last stage of the disease de-hydrocholin was given orally and parenterally and a 25 per cent solution of glucose containing vitamin C (250 mg.), vitamin B_1 (25 mg.) and nicotinic acid (25 mg. to 50 mg.) and 5 c.c. of 30 per cent hexamine solution intravenously. There was, however, little benefit from these at that stage of the disease.

The dietary regimen of the children under treatment were restricted to cow's milk without much cream, rice with soups of greens, butter-milk, fruit juice and barley water. Glucose and sugar were allowed liberally. Fats and fatty foods were strictly precluded. The dietary availability of calories was 1,000 calories per child per day.

Recession of the enlarged liver and spleen and remission of other concomitant clinical symptoms have been taken as criteria of clinical improvement. Clinical recovery has been confirmed by liver-function tests in a number of cases. A case has been considered clinically cured when there is no palpable enlargement of the liver and spleen, the clinical symptoms have been completely relieved and the general condition has been restored to normal. Tables I and II summarize the clinical results :—

TABLE I.

Treatment with choline chloride only.

Pathological condition Stage.	Total number treated.	Number cured.	Number died.	Number discontinued.	Per cent cured.	Period of treatment for effecting cures, in weeks.
I	28	28	...	...	100	3–5
II A	56	56	...	...	100	6–12
II B	23	12	4	...	52.1	9–16
III	16	...	12	...	0	...

TABLE II.

Treatment with liver-spleen hydrolysate supplemented with choline chloride.

I	18	18	...	...	100	2–4
II A	32	32	...	...	100	2–6
II B	21	12	6	3	57·1	8–10
III	22	...	22	...	0	...

Cases which proved resistant to treatment with choline chloride alone in stage II-B received a supplement of the liver-spleen hydrolysate as in the other group, after a trial period of six weeks, or earlier in case grave symptoms appeared. In the above series there were seven such cases. Three of these recovered, while the other four passed on to the next stage and ultimately died. In reckoning the 'per cent cured' in the above group only those which were cured by the administration of choline chloride alone have been taken into consideration.

For the sake of proper comparison the results in the present series have been combined with those obtained previously in the respective groups.

The pathological stage referred to in the above column defines the clinical condition of the patient at the commencement of treatment. The period of treatment has been reckoned from the day on which treatment was begun to the day on which there was no palpable enlargement of the liver.

All recovered cases have been treated with the glandular extract supplemented with choline chloride for periods of 8 to 12 weeks after complete recovery in order to prevent any relapses. This was adopted in the choline-treated group of cases also with a view to economize on the limited stock of choline chloride. A number of cases that were completely cured were—and some still are—under observation for long periods after cessation of treatment.

The following clinical observations are of importance in regard to the therapeutic action of choline chloride and its comparison with that of the glandular hydrolysate. In almost all cases choline chloride relieved constipation, presumably by stimulating intestinal peristalsis. The cholagogic action of choline—as judged by the time taken for the change in the pale colour of the stools to normal—was milder than that of the glandular hydrolysate. The excessive amounts of fat disappeared from the stools in about 2 to 3 weeks in the choline-treated group, while this happened in about a week in the other group. Choline did not appear to stimulate appetite until after a long period of treatment, whereas stimulation of gastric secretion and appetite has been found to be an important therapeutic property of the glandular extract. In the choline-treated group liver-extract therapy had to be invariably resorted to for counteracting anæmia even in the early stages of the disease. On the other hand, the glandular hydrolysate exerted a marked hæmatopoietic action in the early stages of the disease. The general pace of clinical recovery was slower in the choline-treated cases.

Cases which resisted treatment in stage II-B gradually deteriorated developing œdema, ascites and jaundice and finally ending in death.

2. Liver function tests.

The thymol-turbidity, the cephalin-cholesterol-flocculation and the bromsulpholein-retention tests of liver function were chosen for comparative study at different stages of the disease. The icteric index was also determined in a number of cases. The thymol-turbidity tests were carried out according to MacLagan (*Nature*, 1944, **154**, p. 670) using Burroughs Wellcome vaccine standards for the quantitative estimation of turbidity. The cephalin-cholesterol test was performed according to Hanger (*Jour. Clin. Invest.*, 1938, **18**, p. 261) using the standard cephalin-cholesterol preparation supplied by the Wilson Laboratories, U.S.A. The degree of flocculation was estimated visually and was graded in terms of 0, +, ++, +++ and ++++, depending upon the completeness of flocculation of the emulsion. Thus, a reaction of ++++ indicates complete flocculation leaving the supernatant liquid water clear. The bromsulphalein retention and the icteric index were determined according to methods described in Harrison's "Chemical Methods in Clinical Medicine (1944)". The grading in the bromsulphalein-retention test is based on the percentage of the injected dye retained in the serum half an hour after injection; thus grade I 5 to 15 per cent, grade II over 15 per cent, grade III over 25 per cent and grade IV over 40 per cent. The results are given in Table III. The figures in brackets indicate the number of cases.

In each of 19 cases at different stages of the disease all the tests were carried out simultaneously. The results are given in Table IV.

TABLE III.

Pathological stage.	Thymol turbidity reading.	Cephalin-cholesterol flocculation.	Bromsulphalein-retention grade.	Icteric index.
I	6 (21)	+ (2)	I (3)	3 (6)
	5 (10)	++ (9)		5 (2)
	7 (1)			6 (1)
II-A	6 (5)	+ (1)	I (3)	3 (3)
	5 (39)	++ (5)	II (3)	5 (3)
	4 (16)	+++ (3)		10 (5)
II-B	4 (27)	++ (3)	III (6)	5 (2)
	3 (4)	+++ (3)		8 (1)
		++++ (2)		10 (7)
				15 (3)
III	3 (4)	+++ (4)	III (2)	15 (2)
	2 (15)	++++ (4)	IV (2)	25 (3)
				30 (4)
				40 (1)

TABLE IV.

I	5	++	I	...
	6	++	I	...
	5	+	I	5
	5	++	I	6
	5	++	I	5
	5	++	I	3
	6	+	I	10
II-A	5	+++	II	...
	5	++	II	...
	4	++	III	...
II-B	3	+++	III	10
	3	+++	III	15
	4	+++	III	10
	4	++	III	8
	4	++	III	...
III	3	++++	III	...
	2	+++	IV	...
	2	++++	III	30
	2	+++	IV	35

Based on the above and also the previous results it may be stated that the thymol-turbidity test runs more nearly parallel to the pathological condition than the other tests. The cephalin-cholesterol test appears to be hypersensitive and the degree of flocculation is at variance with the pathological classification based on clinical symptoms. Further studies on these tests in this and other hepatic diseases are in progress.

In the thymol-turbidity test the turbidity decreases with the advance of the pathological condition ; stage I 5 to 7, stage II-A 4 to 6, stage II-B 3 to 4 and stage III 2 to 3. A decrease in the turbidity reading with remission of concomitant clinical symptoms is indicative of recovery. A persistent low reading (of 3 and less) denotes unfavourable prognosis. In conjunction with clinical symptoms the test is of both diagnostic and prognostic value.

3. Biochemical findings.

(a) *Fœcal fat analyses.*—The fæces collected individually from children at different stages of the disease, for 3 to 4 days before commencing treatment, was dried and analysed for total fat and fatty acids according to the second Soxhlet method outlined in Harrison's ' Chemical Methods in Clinical Medicine ' (1944). The results are given in Table V :—

Table V.

Pathological stage.	Number of cases.	Total fat—per cent of dry fæces.	Degree of splitting fatty acids as percentage of total fat.
I	14	25—40	75—90
II-A	12	35—50	75—90
II-B	10	40—50	60—70
III	8	50—60	45—55
Recovered cases	9	11—20	75—95

During the experimental period the daily fat intake of the children was 15 g. to 20 g. per day per head mostly derived from milk.

The fæcal fat is distinctly higher than the average normal (30 to 35 per cent of dry fæces) and the percentage of fat in the fæces increases with the advance of the pathological condition. The degree of splitting of the excreted fat—and hence the pancreatic efficiency—is normal excepting in the very advanced stages of the disease. The above results indicate that pancreatic efficiency, with particular reference to lipase, is normal and that defective absorption of fatty acids is the main cause of the excessive excretion of fat.

(b) *Plasma-lipoids cholesterol and lipid phosphorus.*—These plasma constituents were estimated in 21 cases at various stages of the disease. The cholesterol was estimated according to the colorimeteric technique of Myers and Wardell and the lipid phosphorus by Youngburg's method (*see* Harrison's book referred to above and also ' Practical Physiological Chemistry ' by Hawk and Bergeim, 1945).

Table VI.

Pathological stage.	Number of cases.	Total lipoids. (mg. per 100 c.c. plasma).	Cholesterol. (mg. per 100 c.c. plasma).	Lipid phosphorus.
I	4	480— 600	180—240	5·6—6·8
II-A	8	510— 750	180—310	4·0—6·0
II-B	5	520— 900	250—450	3·4—5·2
III	4	1,400—1,800	400—585	2·8—3·9

The present results compared with normal ones indicate that plasma content of cholesterol is elevated and that of lipid phosphorus lowered in this disease. The studies are being continued.

(c) *The plasma non-protein nitrogenous constituents.*—The total non-protein nitrogen (n.p.n.) and uria in plasma were determined in a number of cases.

TABLE VII.

Pathological stage.	Number of cases	Non-protein nitrogen. (mg. per 100 c.c. plasma).	Urea. (mg. per 100 c.c. plasma).
I	6	120—140	15—30
II-A	5	130—160	15—25
II-B	5	70— 90	20—35
III	8	120—140	25—30

The results show that whilst the uria content is within the normal limits even in the very advanced stages the total n.p.n. is very much elevated in this disease.

4. Liver biopsy studies.

Adopting the aspiration-biopsy technique using the Vim-Silvermann needle and taking precautions against possible haemorrhage and other complications, liver biopsies were carried out in five cases—one in stage II-A, two in stage II-B and the others in stage III. Two of these had been treated for three weeks with choline chloride.

Histological examination of the biopsy specimens showed heavy fatty-infiltration into the liver in stage II-A case and varying degrees of fatty degeneration, necrosis and fibrosis in the other cases. In the choline-treated cases the sections showed that the liver had been markedly cleared of excess fat as contrasted with the other cases. Cultures of biopsies specimens did not show the presence of any pathogenic or other organisms.

A detailed report of the histological findings will be prepared and submitted separately.

5. Discussion.

The above clinical results show clearly that choline therapy is effective in the treatment of the disease in its early stages. Supplementation with pepsin-proteolysed extract of liver and spleen is essential for treatment in the more advanced (especially II-B) stages of the disease. In all stages of the disease, the rate of clinical recovery is much quicker with the glandular hydrolysate supplemented with a small amount of choline chloride (140 mg. to 150 mg. per day) than with three times that amount of choline chloride given alone. This augmenting effect of the glandular extract is probably due either to provision of lipotropic factors which act more rapidly than choline or direct stimulation of the liver cells which in turn also facilitates the action of choline and other lipotropic factors.

The failure of the therapeutic treatments adopted in the terminal phases of the disease is probably due to extensive and irreversible fibrosis in that organ which in consequence has lost most of its regenerative and functional capacity.

The modes of treatment have also been ineffectual in arresting the disease in a certain proportion of cases in the pre-icteric II-B stage. So far the best response at this stage of the disease (85 per cent cured) has been to treatment with the liver-spleen hydrolysate supplemented with choline chloride and also casein hydrolysate. This will now be verified by further clinical trials. Anyway, this appears to be the stage of the disease wherein one could hope to reverse the histopathological changes in the liver and obtain almost hundred per cent cure by additions to and suitable modification of the above course of treatment.

In addition to studying the concentration and identification of the active principles concerned in the therapeutic action of the liver-spleen hydrolysate, one should also look for other and even stronger lipotropic agents than choline and also factors which prevent hepatic necrosis and fibrosin.

Some of our own findings and also recent work reported from elsewhere point to gastric tissue as a possible source of a powerful lipotropic factor.

Gastric dysfunction was revealed by hypochlorhydria and low enzyme activity has been observed in children at all stages of the disease, and also in the examined cases of some parents of these children. Certain fractions of bovine gastric mucosa obtained by extraction with acid (0·1 N HCl) and precipitation with ammonium sulphate (50 per cent saturation), are effective in the treatment of the early stages of the disease which present investigation has shown to be also amenable to choline therapy.

Recent work of Billman and Gillman (*Arch. Int. Med.*, 1945, 76, p. 63) shows that 'Ventriculin' (desiccated hog stomach) contains a powerful lipotropic factor. They found that in severe cases of infantile pellagra ventriculin administration dramatically cleared the intensely fatty liver and also relieved the œdema and other associated symptoms.

Preparations from gastric tissue deserve a fair re-trial in this disease, by themselves and as supplemented with other lipotropic factors and protein hydrolysate.

The biochemical findings—excessive excretion of fatty acids, a high plasma n.p.n. content without elevation of the urea level, and low plasma-lipid phosphorus all point to a disturbance of protein, fat and perhaps phosphorus metabolism in this disease. Further studies on the distribution of non-protein nitrogen and phosphorus compounds in plasma and protein, fat and phosphorus balance at different stages of the disease would indicate the nature of these metabolic defects. Phosphorus metabolism deserves careful consideration, especially in view of the excretion of copious amounts of phosphate in urine even in the early stages of the disease.

In view of the clinical results obtained with choline treatment a study of the choline content of the blood would throw much light on the question whether choline deficiency or any defect in its metabolism plays an important part in the pathogenesis of this disease.

That choline chloride, an established lipotropic factor, is efficacious in curing the disease in the early stages is highly significant as it indicates that the pathological lesion of the liver in these stages of the disease is predominantly one of fatty infiltration. Liver-biopsy studies confirm this conclusion.

The excessive accumulation of fat in the liver may be due to : (a) deficiency of choline and B-vitamins or a relatively high fat content in the diet,

(b) liponeogenesis from carbohydrate, (c) metabolic derangement resulting in faulty utilization of protein, fat or even carbohydrate and (d) physiological defects. The problem of the ætiology infantile hepatic cirrhosis must be tackled from these various stand points.

A question germane to the above problem is the rôle that diet may play in the pathogenesis of this disease. Certain considerations show that diet plays an important part in the causation of the disease. It is known for a long time now that the incidence of the disease is highest in vegetarian, particularly rice-eating, communities and that among children fed exclusively on cow's milk. Cow's milk would itself constitute a relatively high fat diet if its protein is not completely and properly utilized. It is plausible that a child inherits a low capacity to utilize fat and protein owing to its predecessors having consumed for generations a low fat, low protein and high carbohydrate diet. When to such a child a fat-rich food, such as cow's milk, is exhibited it could lead to accumulation of fat in the liver. The peculiar physiological changes that may occur during the critical growth period of the child, other concomitant deficiencies in the diet, and also infections would all act as aggravating factors. Persistent fatty infiltration into the liver has been experimentally demonstrated to lead ultimately to fibrosis and its clinical sequelæ. Certain biochemical and histological findings pointed out above show that the metabolism of protein and fat is defective in this disease. At the present state of our knowledge in regard to the disease the above postulate could be used as a helpful working hypothesis subject to verification and modification in important respects on the basis of further findings.

In the light of the foregoing observations it would be of considerable practical significance to ascertain the effect of eliminating or lowering the fat content of the diet on the clinical course and response to treatment at different stages of the disease. If the liver fat accrues primarily from the dietary fat, the response to any form of rational therapy should be accelerated by eliminating fat in the diet and in the early cases should by itself considerably ameliorate the clinical condition. Even in case the liver fat is the result of liponeogenesis from carbohydrate, lowering the fat content should lead to increased pace of recovery under treatment as this would largely reduce the strain on the already impaired liver and perhaps also the digestive organs.

A beginning has already been made in the above matter of therapeutic dietetics in this disease. Soya milk has been selected as a substitute for cow's milk owing to its low fat content, ease of digestibility and as high a biological value as that of cow's milk. It is also reported to be better utilized by children than cow's milk. In addition to observations on clinical response to treatment, protein- and fat-metabolism studies will be conducted in a few select cases. The content and composition of fæcal fat in relation to that ingested through the diet will also be studied.

6. Summary of work done.

(a) Choline chloride is effective in the treatment of the disease in its early—pre-cirrhotic—stages. Supplementation of choline chloride with a pepsin proteolysed extract of the liver and spleen is necessary for efficacious treatment in the more advanced stages. It also hastens the therapeutic action of choline chloride.

(b) A comparative study of the thymol-turbidity, the cephalin-cholesterol and the bromsulphalein-retention tests of liver function has shown that the thymol-turbidity test is a better indicator of the pathological stage of the disease than the others. In conjunction with clinical symptoms, it has been found to be of both diagnostic and prognostic value in this disease.

(c) Biochemical studies have shown that in this disease pancreatic efficiency as judged by the degree of splitting of fæcal fat, is normal, the plasma-lipid phosphorus content is low and plasma n.p.n. level is elevated.

(d) Liver biopsy studies have been conducted in six cases. The liver lesion in the early stages of the disease has been found to be one predominantly of fatty-infiltration. No pathogenic or other organisms were found in these specimens.

6. Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay.

During the period under review a further supply of specific rickettsial antigens was made available through the courtesy of American workers at the National Institute of Health, Bethesda, and Lederles Laboratories, New York. Using these antigens, complement-fixation tests were carried out on sera from typhus cases and also on sera of field and domestic rats in Bombay city. The important rôle of complement-fixation test in the epidemiological study of typhus infection was dealt with in the *Report* for 1947. Attempts were also made to isolate human, rat and mite strains.

(a) *Serological diagnosis.*—This year also, samples of febrile cases were tested for Weil-Felix reaction against the three standard antigens, namely proteus X19, XK and X2. In all 1,495 samples of sera were tested. One hundred and twenty-three samples of 81 cases were positive for X19 type and only 16 samples of 8 cases were positive for XK type. Clinical histories were available in a number of cases, but nothing further could be added to the previoue observations recorded on that basis.

(b) *Isolation of murine strains.*—Twenty-nine attempts were made to isolate murine strains from human cases by inoculation of blood into male guinea-pigs whenever possible or, in their absence, in laboratory-bred white rats. Out of 8 guinea-pigs so used, 2 showed febrile reaction, only 1 showed Neil-Mooser reaction in addition and the rest did not react at all. Sera of these positive and negative reactors were tested for complement-fixing anti-bodies. In one negative reactor, the anti-bodies were clearly demonstrated in the serum, thus suggesting the possibility of " silent infection " in such animals.

(c) *Study of human typhus sera by complement-fixation tests.*—One hundred and twenty sera obtained from 82 cases were put for this test using Bengston's technique. Seventy-six samples derived from 51 cases were positive against murine antigen. Usually the results of Weil-Felix and complement-fixation compared favourably but few cases were encountered in which the results did not agree.

The following table illustrates the point :—

Serial number.	Wital number.	Weil-Felix titre :			Results of complement-fixation test.
		X19	X2	XK	
1	219	1,600	...	...	Negative.
2	229	800	...	...	,,
3	338	400	...	...	,,
	366	1,600	...	...	,,
4	358	800	...	...	,,
5	367	800	...	...	,,
6	378	1,600	...	...	,,
7	391	1,600	...	...	,,

(d) *Complement fixation test with sera of wild rats.*—As previously reported, 3 species of Bombay rats were taken for study ; *Rattus rattus*, *Rattu norvegicus* and *Gunomys*. A fourth species, *Suncus cerulens*, has been added this year. Three hundred and fifty-five rat sera were examined during the period under review and, as expected, *Rattus norvegicus* showed the highest percentage of positive infection for murine typhus. Last two years findings regarding the murine typhus infection amongst the different rat population of Bombay city is summarized below :—

Table showing the results of complement fixation test on rat sera for two consecutive years.

Species examined.	Total number examined.	Positive complement-fixation test.	Percentage positive.
R. rattus	323	62	19·2
R. norvegicus ...	193	84	45·0
Gunomys	208	13	6·2
Suncus cerulens ...	15	0	0
	739	159	24·22

(e) *Isolation of murine strains from Bombay rats.*—Isolation of murine strains from Bombay rats such as *R. rattus* and *R. norvegicus* have already been reported in previous report of this enquiry. During the year, a new species of rats, *Suncus cerulens*, was available for examination in small numbers. Their sera were tested by complement-fixation test. As shown in the above table, all the sera of this species were negative by this test. Eight brain-pooled suspension from 23 rats of this species were inoculated in the guinea-pigs. Four guinea-pigs showed regular febrile reaction, but no strain has yet been isolated. Further work regarding this is in progress.

(f) *Survival of murine virus in laboratory-bred white rats.*—This experiment was planned last year to throw some light on the epidemiology of typhus infection in Bombay. Two laboratory-bred white rats were inoculated with two different human strains of murine virus and the third one was inoculated with a rat strain of the same virus, and were kept under observation. Brain of each

rat was passaged separately into three male guinea-pigs at the end of 472, 510 and 522 days respectively. All the three guinea-pigs showed febrile reaction. Neil-Mooser reaction was observed in one and the complement-fixation test was positive in two. This experimental observation, though solitary one, clearly establishes the fact that murine virus is capable of surviving for a long period of over a year in white rats and this very probably holds good for wild rats too. Pollard and Auguston (1945) in U.S.A. have also reported that the murine typhus agent could be isolated from the brain of rat as long as 370 days after inoculation. Thus the wild rats once infected in nature, maintain such infection for a very long time and thus act as reservoirs of this infection.

(g) *Experiment conducted to ascertain the possibility of transmission of murine virus infection from the infected parents to their offsprings.*—A male and a female white rat after being infected with the murine virus (Wilmington strain) were kept together in the same cage for breeding. After about 82 days the female produced a litter of three. When the young ones were 22 days old, the parents and the two young ones were killed and heart-blood collected. Three guinea-pigs were separately inoculated with the brain suspensions of each lot. The following table illustrates the results :—

	Complement-fixation.	Guinea-pig :		
		Febrine reaction.	S.S.	Complement-fixation.
Male	+	+	−	+
Female	+	+	+	+
Young male	+	+	−	−
Young female ...	+			

The results obtained are very suggestive of the transmission of virus to the offspring but they require to be confirmed by repeated observations. Further work in this subject will throw more light on the endemic nature of typhus infection.

Work on scrub typhus.

Although sera from febrile cases were examined all the year round for presence of XK typhus, it was only after monsoon a few cases were detected serologically. Sixteen samples derived from 8 cases showed titres varying from 50 to 3,200, but only three cases showed titres above the diagnostic level. The number of such cases being very small, mice inoculation was done in all cases but no strains could be isolated. The three serologically positive cases occurred in the month of November 1947.

Trombiculid larvæ were collected from different species of rats, the total number being 2,628. One thousand five hundred and fifty-eight were picked up from *Gunomys*. 990 from *Rattus norvegicus* and only 50 from *Rattus rattus*. The small number of *Suncus ceruleus* examined showed no mite infestation. Some of these larval mites were sent to Dr. Krishnan, All-India Institute of Hygiene & Public Health, Calcutta for identification, and 2 species, *Tr. indica* and *Tr. paltai* were reported by him. Another lot was identified as *Asco-schongastica indica hirst*. One more lot has been sent to the National Institute of

Health, Bethesda, U.S.A. and the report is awaited. Attempts were also made to isolate *R. orientalis* strains from mites. Five different pools of larval mites varying from 40 to 80 in each pool were inoculated into white mice, but no strain has been so far isolated. In view of the small number of XK typhus cases and with peculiar short-term seasonal incidence, it is difficult to locate the arthropod vector unless the work is carried on from year to year.

7. Enquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

Since the last report submitted in December 1917, the following investigations have been conducted :—

Clinical features of XK typhus.—Three hundred and fifty-two cases of fever were investigated in the Barrackpore-Ichapore area. The laboratory studies that were conducted on the blood of fever cases revealed that among these there were 58 cases of typhoid, 16 cases of paratyphoid, 22 cases of kala-azar, 5 cases of malaria and 72 cases of typhus. The rest could not be diagnosed. The diagnosis of typhus cases was based on clinical findings, Weil-Felix reaction and isolation of rickettsia in laboratory animals—guinea-pigs and mice. The clinical features of these cases were recorded in a schedule prepared for the purpose. Analysis of the data pertaining to cases of XK type which is the predominating type in this area showed that the cases may broadly be grouped into two clinical types—*severe* and *mild*. About 60 per cent belonged to the mild type and about 20 per cent to the severe type. The mild type was characterized by fever, 100°F. to 102°F., lasting for 8 to 12 days, pains in the muscles and headache. There was no rash or eschar. Glandular enlargement and injection of eyes were only occasionally met with. Mortality was nil. Diagnosis of these cases on clinical grounds without laboratory aid was impossible. Weil-Felix reaction gave a significant titre (1 in 125 or above) in only about 65 per cent of cases and that too after repeated examinations of blood (at least twice) in the course of the illness. In the serologically negative cases animal inoculation (into white mice—Haffkine Institute strain) proved positive in 72 per cent. Using both serological and animal inoculation tests nearly 85 per cent of cases could be diagnosed. The severe type was characterized by fever—120°F. to 104°F., for 14 to 20 days. A macular rash appeared on or about the 5th day in 43 per cent and disappeared in 24 to 48 hours after its appearance. Eschar was present in 36 per cent of cases. The eschar was either a papule or a necrotic ulcer. Both types of eschar were equally frequent. Injection of eyes, glandular enlargement, persistent headache and insomnia were quite frequent. Case fatality was about 14 per cent. It was possible to arrive at a tentative diagnosis of typhus in nearly 50 per cent of severe cases on clinical grounds. This was invariably confirmed by serological and animal inoculation tests. The severe type was more often seen in new comers and the mild type in the indigenous people. There were more male than female cases and all cases were adults.

Value of the Weil-Felix test in typhus.—In all suspected cases of typhus the blood was obtained and tested for the Weil-Felix reaction. Suspensions of OX19, OXK and OX2 were used in all cases. These suspensions were prepared and standardized in the laboratory and tested for sensitivity before use. Of the 62 cases diagnosed serologically 46 were XK, 12 were X19 (murine) and 4 were X2(?).

No difficulty was experienced in diagnosing the X19 typhus cases by this test. The positive bloods gave good agglutination in high titres. A titre of 1 in 500 to 1 in 1,250 was obtained in 70 per cent and in 30 per cent the titre was between 250 and 500. Often one examination was found sufficient. In four of these cases in which male guinea-pigs were inoculated with crushed blood clot the Neil-Mooser reaction was obtained. Animal inoculation was not found necessary for establishing diagnosis as the serological results were fully dependable.

In the case of XK typhus which was the predominating type of disease agglutination in high titres was less frequently obtained. In 5 per cent it was below 1 in 50, in 20 per cent the titre rose to 1 in 50 only, in 30 per cent it was over 1 in 125, in 25 per cent it was over 1 in 250 and in 10 per cent it was over 1 in 500. In the last group the highest titre recorded was 1 in 1,250. Although in a fair percentage of cases an increase in titre to the diagnostic level occurred about the 10th day, it was noticed much later in most others. In some cases the titre fluctuated in the course of disease without rising much and in some others the diagnostic level reached only near about the crisis or during convalescence. In 10 per cent which were proved positive by animal inoculation, the serum gave negative agglutination throughout the illness. Both severe and mild cases showed these serological features. Thus, in only about 65 per cent of the cases could one get a significant agglutination titre by the Weil-Felix reaction.

The diagnostic significance of OX2 positives is not clearly known. In the four cases that gave OX2 positive serologically, the highest titre obtained was never over 1 in 125 for OX2 and there was always associated with it agglutination with OXK and OX19, though in a slightly lesser titre. In one case rickettsia were found in the peritoneal scraping of the mouse that was inoculated with washed blood cells. This strain was accepted as one of *R. orientalis*. Though workers in other areas have also reported cases of OX2 positive detailed investigations of these cases have not been conducted so far to our knowledge. The three cases of OX2 referred to above unfortunately were not investigated. It is believed that if guinea-pig inoculation with washed cells had been resorted to, significant results would have been obtained. Our impression is that these X2 cases which occur in this area in spring need to be investigated from the ætiological and epidemiological points of view. We expect to do this when the next crop of cases are detected during next spring.

Value of animal inoculation in the diagnosis of XK typhus.—It is known that Swiss white mice are very susceptible to *R. orientalis* infection when they are injected intraperitoneally with infective material. The animals usually succumb to the infection in about 10 days. In the course of this study mice were used in the diagnosis of clinically positive and serologically doubtful or negative cases. One c.c. of washed blood cells was injected intraperitoneally and it was found that in 72 per cent of cases tested rickettsias could be deomonstrated in the peritoneal scrapings between the 5th and 10th days of inoculation. By the routine use of animal inoculation in doubtful and sero-negative cases, diagnosis can be conclusively established in those cases of XK typhus which would otherwise have been missed. As it has been found that none of the sero-positive cases tested by animal inoculation gave a negative result and that 72 per cent of sero-negative cases gave positive result by animal inoculation, the animal inoculation method is considered superior to serological examination the routine diagnosis of XK typhus.

Maintenance of rickettsial strains.—Six strains of *Rickettsia orientalis* isolated from cases are being maintained in mice. They are now in the 40th to 50th passage. Passage through mice increases virulence of strain for guinea-pigs in which ordinarily *R. orientalis* causes only an apparent infection. Passage of these rickettsias through chick-embryo enhances its virulence to mice. Those animals which ordinarily develop symptoms and die between the 8th and 12th days, die within 5 to 6 days instead.

Cultivation of rickettsiae in yolk sac.—Fertile eggs after 6 days incubation at 37° C were used. Six strains (HP_2, HP1A, R216, HK_1, HC and HD) that were being maintained in Swiss white mice by intraperitoneal inoculation were used to cultivation experiments. The material for injection into yolk sac was obtained from infected mice. Suspensions of (i) spleen, (ii) brain, (iii) spleen and ascitic fluid combined, (iv) ascitic fluid alone, all of which showed rickettsia were used for inoculating into yolk sac. The suspensions were made with the following solutions sterilized by steam: (a) Normal saline, (b) phosphate buffer (pH 7·6), (c) starch solution (0·5 per cent in distilled water), (d) 1 per cent, 2 per cent, 5 per cent and 10 per cent solutions of dehydrated skimmed milk in distilled water, and (e) 1 per cent casein in distilled water. The amount of the suspension inoculated was 0·2 c.c. and the strength of suspension was 10 per cent. The inoculated eggs were incubated at 37° C and 35° C. for 96 hours. They were incubated at 37° C. for 10 days when the suspension was made with skimmed milk and centrifugalized and the supernatant used as inoculum. For transfer from egg to egg the yolk sac was used. The yolk sac suspension was also injected into mice at the same time to test its infectivity.

Of the strains used HP_2 and R216 gave the best results as they could be maintained up to 5th generation in yolk sac. Combined spleen and ascitic fluid gave better results than brain or spleen emulsion. The vehicle in which the infective material was suspended, had little influence on the results (except starch solution). Starch solution killed the embryos in 24 hours of inoculation. Incubation at 37° C after inoculation was found to be better than 35° C. probably because embryos remained alive for longer periods at 37° C. The embryo could be kept alive up to a maximum period of 10 to 12 days by reducing the number of rickettsia in the inoculum. This was done by using the supernatant portion after centrifugalization. Passage through egg sac increased the virulence of the strain to mice. Rickettsia were observed on three occasions only in the yolk sac. On the other occasions the yolk sac suspension was infective to mice though smears did not show any rickettsia. After 4th or 5th subculture the embryos began to die within 2 days of inoculation. Neither the yolk sac nor the dead embryos were found infective to mice. Investigation is proceeding to find out the cause of death of embryo after 5th transfer. Cultures of dead embryo have so far proved sterile. The lethal agent is transmissible from yolk sac to yolk sac. The possibility of a virus being involved is being investigated.

Rodents and trombicula mites.—Having already established the fact that *T. deliensis* is the predominating *Trombiculid* in the endemic area, efforts were made to collect large numbers of these mites for breeding purposes. The number and species of rodents collected each month and the number of mites found on them are given below :—

Months.	Rodents captured.	Number infected.	Number harbouring *T. deliensis*.
January ...	14	2	...
February ...	7	1	...
March ...	34	...	...
April ...	43	15	1
May ...	71	24	15
June ...	55	14	13
July ...	73	39	34
August ...	89	36	33
September 1st-8th	24	17	15
Total ...	410	148	111

During the months of April, May and June the number of *Trombiculid* mites found on the rats was small (about 2 to 10 per rat) but with the advent of the monsoon heavier infestations were present (100 to 150 per rat) and a sufficient number of *T. deliensis* larvæ was obtained for breeding. The species of rodents collected were : *R. rattus*, *B. indica*, *G. varius*, *C. cærulea* (shrews). *T. deliensis* was most prevalent on *R. rattus* and shrews. In *R. rattus* the mite larvæ are found in the ears generally and in shrews they are found on the outer side of the limbs and round the anus and genitals.

The study of the ecology and bionomics of *T. deliensis* under natural conditions had to be abandoned owing to the depredations of mongoose, snakes and jackals which not only injured or killed the experimental rats and mice left in the field but also destroyed their cages in their endeavours to get at the caged animals. The conditions best suited to the breeding of this species of mite had, therefore, to be studied in the laboratory under artificial conditions.

Breeding of T. deliensis.—Last year it was reported that nymphs of *T. deliensis* were attracted by silt. Though various methods were adopted to enrich the silt with bacteria and protozoa in the hope that the nymphs would feed on these, nymphs failed to develop into adults. At the close of the season the writer suggested, after discussion with foreign workers, that nymphs might feed on eggs of certain insects and mosquito eggs might be suitable. When the season began this year nymphs were given mosquito eggs and newly hatched larvæ of *Culicines* and it was soon found that they fed and developed well on this diet and grew into adults. The adults also fed on the same type of food as the nymphs. No attempt has yet been made to determine whether the nymphs and adults feed on the eggs themselves or on the newly-hatched larvæ or both. Anyhow this observation fits in nicely with the epidemiology of mite typhus. Now that the laboratory breeding of mites has been successfully accomplished this year, all that remains to be done is to prove the mode of transmission.

The details of the method of breeding are as follows : Rats infested with larval mites are housed in small fine-mesh cages and suspended over a dish of water. As the larvæ get engorged they drop off the rat into the water and can be easily collected from the water on which they are found floating. The larval mites are collected on a small piece of moist filter-paper using a fine brush. The paper is inserted into a tube containing very fine moist sand. The larvæ

get on to the sand and become quiescent in anything from 12 hours to 3 days. They remain in this nymphochrysalis stage for about 7 days and then become nymphs. Newly emerged nymphs are pale-creamy coloured and slender. They feed and grow rapidly in size and assume a distinct pink colour. After feeding for from 7 to 10 days they assume a second chrysalis stage, i.e. the imagochrysalis out from this, after about 7 days, the adult emerges.

Adults may be recognised by the presence of certain folds in the integument of the dorsum, as well as by the presence of the fully developed genital organs. The sexes can only be determined after the mite has been cleared and mounted. In tubes containing newly-hatched adults, larval mites were seen after 20 days. The adult *Trombiculid* mite appears to lay a few eggs at a time over a long period. So far as our observations go they keep on laying eggs for at least three months. Some of the adults that hatched out in July 1948 are still alive and quite vigorous (September 1948) though the number of larvæ found in the tubes has diminished.

Larvæ of *Trombiculid* mites are very minute and can only be seen with a lens. They are very active and climb up the sides of glass tubes and walk on the surface of water. Tubes producing larvæ have therefore to be secured in a container protected with a vaselin block, in addition to a solution of disinfectant. The laboratory-bred larvæ are now actually being fed on infected mice for use in transmission experiments.

Feeding of larval mites.—A certain amount of success has been obtained in feeding the laboratory-bred larvæ on rodents. The technique adopted is as follows: A mouse is taken and enclosed in a well-fitting wire-mesh cage and suspended over the open mouth of the tube containing larvæ. Larvæ climb up the sides of the tube and attach themselves to any part of the skin of the mouse. After they have fed they drop off and are picked up and put into breeding tubes for enabling them to grow into nymphs and adults. Larval mites successfully fed in the laboratory are now in the nymphal stage. It is hoped that they will become adults in due course and their progeny (larvæ) will be available after the middle of October for use in transmission experiments.

Transmission Experiments.

Now that a successful method of breeding mites has been developed, transmission experiments should not present much difficulty. Both naturally infected and artificially infected larvæ are being bred out and the first generation of larvæ coming out of these will be used in these experiments. Already a certain number of rats showing heavy infestation with *T. deliensis* have been caught from known infected locality and dealt with as follows: The larval mites on them were allowed to drop off and then collected and put into breeding tubes. Their progeny when obtained will be used in transmission experiment. The rats were sacrificed and their brain emulsion injected into mice to find out if they were infected with rickettsia. If any of the rats prove to be infected, the progeny of the mites which had fed on these rats would be used in transmission experiments.

Larval mites which have hatched out in the laboratory have been fed on laboratory-infected mice and the progeny of these when ready will be used for feeding on clean mice. These experiments are now in progress.

8. Enquiry on the effects of vitamins C and B in diabetes under Dr. S. Banerji at the School of Tropical Medicine, Calcutta.

Vitamin C and diabetes

It has been observed by us that the deficiency of vitamin C in guinea-pigs leads to diminished glucose tolerance, diminished deposition of liver glycogen and diminished insulin content of the pancreas. It has also been observed that vitamin-C deficiency causes de-granulations in the beta cell of the islands of Langerhans in the pancreas of guinea-pigs. Like guinea-pigs human beings cannot synthetize vitamin C and a chronic deficiency of vitamin C might cause de-granulations and degenerative changes in the islands of Langerhans resulting in diabetes mellitus. The vitamin-C nutrition of diabetic patients and the effect of administration of vitamin C on the glucose-tolerance test and on the urinary excretion of sugar of diabetic patients have, therefore, been studied.

Vitamin-C nutrition of diabetic patients.—The 24 hours' urinary excretion of ascorbic acid of 20 diabetic patients and 5 normal subjects was estimated. All subjects were fed synthetic ascorbic acid in the dose 5 mg. per pound body-weight for two to four days and the 24 hours' urinary ascorbic acid was also estimated. The results are given in Table I :—

Table I.

24 hours' urinary excretion of ascorbic acid both before and after administration of vitamin C for varying periods.

	24 hours' urinary excretion of ascorbic acid in mg. :				
	Before feeding ascorbic acid.	After administration of ascorbic acid :			
		1st day.	2nd day.	3rd day.	4th day.
Diabetic patients (16)	13·2±3·2	33·0±6·7	58·8±20·6	132·1±26·9	178·2±47·6
Normal subjects	15·6±3·6	288·0±67·0	349·0±92·0	...	...
Difference of means ...	2·4	255	290	...	...
Standard error of difference	4·9	67·4	94·7	...	...
t ...	0·49	3·78	3·06	...	...

The normal subjects excreted significantly increased amounts of ascorbic acid when fed ascorbic acid than diabetic patients. Urinary excretion of ascorbic acid before the administration of ascorbic acid did not differ significantly in the diabetic and normal subjects. The results indicate that diabetic patients do suffer from vitamin C deficiency.

Effect of vitamin C on the glucose tolerance test of diabetic patients.—Diabetic patients, when admitted in the Carmichael Hospital for Tropical Diseases, were given a diet consisting of protein 135 g., fat 90 g. and carbohydrate 180 g. This diet roughly furnished 2,000 calories and the patients took

this diet during their stay in the hospital. After the patients received this diet for one week, the oral glucose-tolerance test was performed. After the glucose-tolerance test they were fed ascorbic acid daily in the dose 5 mg. per pound body-weight for a period of three weeks. The glucose-tolerance test was again performed. The results are given in Table II :—

TABLE II.

Oral glucose-tolerance test of sixteen diabetic patients.

	Mg. glucose per 100 c.c. blood :				
	0 hour.	½ hor.	1 hor.	1½ hours.	2 hours.
Before ascorbic acid ...	143 ± 10	206 ± 13	256 ± 13	271 ± 14	243 ± 16
After ascorbic acid ...	113 ± 5	177 ± 8	230 ± 12	231 ± 11	204 ± 11
Difference of means ...	30	29	26	40	39
Standard error of difference ...	11·7	15·5	17·9	17·8	19·8
t ...	2·56	1·8	1·45	2·24	2·0

The fasting blood-sugar level of diabetic patients after the administration of ascorbic acid was significantly lower than the value obtained before the ascorbic acid was fed. The difference in the rise of blood sugar did not vary much up to one hour after the feeding of glucose. The blood-sugar values after one and one half and two hours after the feeding of glucose were significantly lower when the patients received ascorbic acid. This indicated that the utilization of glucose was slightly improved in the diabetic patients when they received ascorbic acid for three weeks. The nature of the glucose-tolerance curves, however, remained to be diabetic even after the administration of ascorbic acid.

Effect of vitamin C on the urinary excretion of sugar by diabetic patients.— The diabetic patients after admission into the hospital received the diet already mentioned. Twenty-four hours' urinary excretion of sugar of the patients was determined daily. The diet fed considerably lowered the urinary excretion of sugar of these patients. After a week when the effect of diet on the urinary excretion of sugar was more or less constant the patients were fed ascorbic acid in the dose mentioned above. The urinary excretion of sugar was estimated daily. In ten patients the urine was sugar-free three weeks after the administration of ascorbic acid. In some cases, however, ascorbic acid could not lower the urinary excretion of sugar but when insulin was injected the urine became sugar-free.

The above results indicate that diabetic patients do suffer from vitamin-C deficiency. They, therefore, should get more ascorbic acid in their diet. The fasting blood sugar of most of the diabetic patients came to the normal level after they were fed ascorbic acid and some of the diabetic patients did not excrete sugar in their urine after receiving ascorbic acid. The higher tolerance of glucose after the administration of ascorbic acid is also suggestive that the pancreas secretes more insulin after ascorbic acid. If the beta cells of the islands of Langerhans are already dead vitamin C may not have any action on those cells. This explains the failure of ascorbic acid to lower the urinary excretion of sugar in some diabetic patients.

Vitamin C and thyroid.

It has been reported that patients with hyperthyroidism have high blood-sugar and show a hyperglycemic reaction after glucose. These are reduced after thyroidectomy. It has also been observed that in diabetic patients total thyroidoctomy increases the carbohydrate tolerance and reduces the insulin requirements. The glycogen value of the liver of animals is decreased when experimental hyperthyroidism is produced. The injection of thiouracil, an antithyroid agent, has been found to cause a definite increase in the glycogen value of the liver in guinea-pigs. As it has also been observed by us that scorbutic guinea-pigs show a diminished sugar tolerance and diminished glycogen value of the liver, the metabolism of thyroid gland might be interfered with during the development of scurvy. The effect of thyroid gland and antithyroid agents on the altered carbohydrate metabolism in scurvy have, therefore, been investigated in guinea-pigs.

Six groups of guinea-pigs were placed on six different diets for a period of three weeks. The diets were: (1) Scorbutic diet only consisting of ground oats 64 parts, ground gram 20 parts, casein 12 parts, calcium carbonate 3 parts and salt 1 part; (2) scorbutic diet containing 1 per cent methyl-thiouracil; (3) scorbutic diet with 0·05 per cent thyroid; (4) scorbutic diet with a daily supplement of 5 mg. of vitamin C per animal; (5) scorbutic diet with methyl-thiouracil with a daily supplement of vitamin C and (6) scorbutic diet with thyroid with a daily supplement of vitamin C. The animals of all the groups were weighed twice a week. Glucose-tolerance test was performed on the 22nd day of the experiment in all the animals of the different groups. The results are given in Table III:—

Table III.

Glucose tolerance test in guinea-pigs on different diets.

Guinea-pigs on :	Fasting blood-sugar.	Blood-sugar after feeding glucose :			
		45 minutes.	90 minutes.	135 minutes.	180 minutes.
	mg. per cent.	mg. per cent.	mg. per cent.	mg. per cent.	mg. per cent.
Scorbutic diet only (8)	150±2	326±23	333±22	306±31	251±37
Scorbutic diet with methyl-thiouracil (8)	151±10	298±23	316±31	333±24	266±37
Scorbutic diet with thyroid (8) ...	151±5	331±3	317±17	251±17	210±16
Scorbutic diet with vitamin C (8) ...	132±5	279±11	251±17	194±14	136±4
Scorbutic diet with vitamin C and methyl-thiouracil (8) ...	121±5	261±10	256±8	196±12	146±14
Scorbutic diet with vitamin C and thyroid (8)	147±5	271±10	262±17	210±20	170±33

The type of glucose-tolerance curves of the three groups of guinea-pigs, which received scorbutic diet, scorbutic diet with methyl-thiouracil and scorbutic diet with thyroid, was more or less similar. The blood-sugar values both before and for varying periods after the feeding of glucose did not vary significantly in these animals. This indicated that the deficient utilization of glucose as observed in animals with scorbutic diet only was not affected by either hypo- or hyperthyroidism. The remaining three groups of guinea-pigs which received the three diets mentioned above with the addition, however, of a daily oral supplement of vitamin C behaved differently. The blood-sugar level, three hours after feeding glucose, in guinea-pigs receiving thyroid gland was significantly higher than the blood-sugar level in guinea-pigs receiving no thyroid gland. This indicated that hyperthyroidism lowers the carbohydrate tolerance in guinea-pigs. Methyl-thiouracil, however, had no effect on the utilization of glucose.

Guinea-pigs which received thyroid gland in their diet began to loose weight from the day they were fed the diet. The feeding vitamin C had practically had no effect in preventing the loss in weight of these animals. The growth rate of guinea-pigs receiving methyl thiouracil, with or without the supplement of vitamin C, was comparable with the guinea-pigs receiving scorbutic diet only with or without the supplement of ascorbic acid.

The altered carbohydrate metabolism as observed in scorbutic guinea-pigs is, therefore, not due to either hypo- or hyperthyroidism.

Vitamin B_1 nutrition of diabetic patients.

Contradictory claims have been made with regard to the effect of vitamin B_1 in the treatment of diabetes. Vitamin-B_1 nutrition of diabetic patients was, therefore, studied. 24 hours' urinary excretion of vitamin B_1 was determined in eight diabetic patients. All the patients then received an oral dose of 5 mg. of vitamin B_1. Urine was collected 4 hours after the feeding of vitamin B_1 and again after 24 hours. The results are shown in Table IV :—

Table IV.

Urinary excretion of vitamin B_1 in gamma.

	Before test dose.	After test dose of vitamin B_1 (5 mg.).	
	24 hours' excretion.	4 hours' excretion.	24 hours' excretion.
Number of patients (8) ...	300 ± 38γ	222 ± 32γ	632 ± 58γ

The above results indicate that the vitamin-B_1 nutrition of the diabetic patients studied are normal. In experiments with animals it has been observed that the insulin content of the pancreas of rats is not diminished when the rats develop vitamin-B_1 deficiency. Vitamin B_1, therefore, does not seem to have any rôle in diabetes.

9. Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

I. Studies with vegetarian blood.

Subjects of study.—These were selected from among healthy adults who were strictly vegetarians from their birth. Their ages varied from 17 to 50 years. Altogether 109 subjects were studied, consisting of 89 males and 20 females. Excepting four subjects who belonged to the Jain community, all were from South India and the majority belonged to the middle class.

(a) *Blood picture.*—Complete cytological examination of blood, including hæmatocrit reading, erythrocyte sedimentation rate, Arneth count and van den Bergh test was done in all the cases according to standard methods ('Hæmatological Techinque' by Napier and Das Gupta, 3rd ed., 1945). The details of the different values are given below separately for males and females. According to the standard in the non-vegetarians, slight anæmia was noted in 5 females and 1 male. The anæmia was macrocytic in type in all these cases.

	Male (89 cases).		Female (20 cases).	
	Mean.	Standard deviation.	Mean.	Standard deviation.
Hæmoglobin in g. per 100 c.c. ...	16·69	1·29	13·63	1·66
Red cells in million per cu. mm. ...	5·22	1·33	4·24	0·57
Reticulocyte percentage ...	0·23	0·92	0·26	0·16
Mean corpscular volume in cu. mm.	88·73	6·61	94·09	0·02
Mean corpscular Hb. in γγ ...	32·01	3·13	32·26	4·17
Mean corpscular Hb. conc. in percentage	35·92	2·19	34·18	2·48
White cells per cu. mm. ...	7,717	2,539	7,197	1,805
Neutrophil ,, ,, ,, ...	4,011	1,689	3,685	1,893
Lymphocyte ,, ,, ,, ...	1,453	74	1,744	854
Monocyte ,, ,, ,, ...	366	244	386	206
Eosinophil ,, ,, ,, ...	1,202	1,854	1,012	868
Basophil ,, ,, ,, ...	36	48	48	67
Arneth count (wt. mean) ...	1·49	0·18	1·44	0·14
Erythrocyte sedimentation rate (Wintrobe 1 hour reading) ...	9·4	7·1	24·0	11·0

A critical examination of the difference data shows that the blood picture closely resembles the blood picture of healthy non-vegetarian Indians except in the Arneth count, eosinophil counts and occasional presence of abnormal cells.

Arneth count.—A comparative figure of the details of Arneth count as found by Das Gupta in his series of normal non-vegetarian Indian males (1941) and the present series is shown below :—

	I Mean.	II Mean.	III Mean.	IV Mean.	V Mean.	Wt. Mean.
Non-vegetarian Indian (male) (Das Gupta, 1941)	37	44	16	3	0	1·85
Vegetarian Indian (male) (present series) ...	58·83	33·3	6·84	0·82	0·04	1·49

The figures of the present series show a preponderance of the cells of class I with lævo-deviation.

Eosinophilia.—In 33 cases of this group total eosinophil count per cu. mm. of blood was above 1,000. In two of the cases with high eosinophilia a few eosinophilic myelocytes were also seen in the peripheral blood. In this series stool was examined in 12 cases only of which hookworm ova was found in 2 cases and *Ascaris* ova in 1 case; in the other nine cases no ova was found in the stool.

In the whole series stool samples of 33 cases were examined for the presence of helminthic ova of whom as many as 11 showed ova of different helminths, viz. hookworm 3, *Ascaris* 1, *Trichuris* 4, *Oxyris* 1, hookworm and *Ascaris* 1, *Ascaris* and *Trichuris* 1.

Nocturnal blood was examined for the presence of *microfilaria* in 13 cases and in two of these *microfilaria* was found in the blood.

Abnormal cells.—Occasional abnormal cells were found in the peripheral blood in 12 cases. These consisted of neutro-myelocyte in 4 cases, eosinophil myelocyte in 2 cases and Turk's cells in 6 cases.

(b) *Van den Bergh test.*—This test was done in all the cases. The test was direct negative in all the cases. The maximum bilirubin content of plasma in this series was 0·6 mg. found in one case only. The plasma bilirubin content was less than 0·6 mg. in the rest.

(c) *Blood-protein level.*—Total protein and albumin : globulin ratio of the plasma was determined by the micro-Kjeldahl method. The difference values are shown below :—

	Male.		Female.	
	Mean.	Standard deviation.	Mean.	Standard deviation.
Total protein ...	6·69	0·49	7·10	0·59
Albumin	4·12	0·64	4·26	0·47
Globulin	2·55	0·66	2·84	0·51

The values were similar to those obtained in normal Indian non-vegetarians.

(d) *Serum-iron level.*—This was estimated in 15 cases, 12 males and 3 females by Powell's modification of Heilmeyers method. The mean value for males was 114 microgram and for females was 83 microgram. These values did not show any significant variation from the values obtained in a few cases of non-vegetarian Indians.

II. *Studies on plasma-protein in anæmias.*

Total plasma-protein and albumin : globulin ratio was estimated by micro-Kjeldahl method altogether in 103 cases of anæmia, before and during the course treatment of anæmia. These cases were comprised of 89 cases of macrocytic anæmia and 14 cases of hypochromic anæmias.

In macrocytic anæmia.—Definite lowering of total protein (below 6·0 g. per cent) was found in 52 out of 89 cases examined. In 50 per cent of the cases with hypoproteinæmia, the albumin fraction was found to be greatly reduced with alteration of the albumin: globulin ratio.

In hypochromic anæmias.—Hypoproteinæmia was found in 7 out of 14 cases in this group. In 80 per cent of these cases the albumin : globulin ratio was altered due to the great reduction of the albumin fraction.

In both groups along with clinical and hæmatological improvement with proper hæmatinics, plasma-proteins also gradually attained the normal level and ratio. Exception was, however, found in one case where the behaviour of protein level was of opposite nature. In a case of severe macrocytic anæmia showing satisfactory hæmopoietic response the plamsa-protein level contrary to expectation gradually went down with great reduction of albumin fraction.

II. Studies in eosinophilia with special reference to the pH of blood.

The CO_2 combining power of plasma was determined according to van Slyke's method in eight cases of the vegetarian group with high eosinophilia and 2 cases of eosinophilic lung. The mean value in these cases was 52·2 c.c. per 100 c.c. In another series of 14 normal cases with normal eosinophil count the mean value was 56·2 c.c. per 100 c.c. Further works in this line are in progress.

10. Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Gastrodiscoides hominis, an intestinal fluke of man and pigs is fairly common in some parts of Assam and the North-Eastern Frontier of India. This worm causes considerable ill-health especially in children, and the object of the enquiry was to find out its life-history to enable adoption of suitable preventive measures. This enquiry was started in 1945.

As the pigs in the Calcutta area were known to harbour this worm, it was decided in the first instance to study the infestation in the pig. The adult flukes and their eggs were collected from the intestine of pigs killed in the slaughter house and brought to the laboratory. The eggs hatched in water beginning from fifteen days to three months, and 143 local snails were exposed to the freshly hatched *miracidia* as reported before. No *cercariæ* developed in any of the snails. It was then decided to carry on the experiments on Assam snails. In July 1946, an assistant research worker was appointed and given suitable training before proceeding to Assam. The rainy season in Assam ends about the end of September, and by previous experience, this time was found suitable for collection of snails and the water-plants. Unfortunately, owing to the serious disturbances in Bengal, it was not possible for the worker to leave Calcutta before the third week of November. Water had already receded from the low-lying areas and the water-plants had become scarce. However, the available snails (which were mostly hibernating) were exposed to the *miracidia* hatching from eggs collected from infested individuals. The *miracidia* did not develop in the snails. The available edible water plants were also examined for *metacercariæ* with negative result.

Continuous study of the problem in the endemic area (at Barpeta) was rendered impossible due to political disturbances. Owing to the influx of a large number of people from outside, the housing problem for the staff became acute, and, thus the work suffered to a great extent. To continue the work, a supply of eggs of the worm from man and pigs and a batch of unexposed

snails were brought down to Calcutta, and a colony of Assam snails was maintained. Exposure of these snails to the *miracidia* was also futile. In 1948 the work was again started at Barpeta. In addition to exposing more snails to the *miracidia*, one thousand eight hundred and seventy snails caught at random were dissected for the purpose of finding out if any *distome cercariæ* are passed by the snails. But this experiment also proved negative. The number of snails dissected is as follows:—

Acrostoma variabile	...	...	31
Gyraulus convexiusculus	...	...	690
Limnæa luteola ...	...	...	127
Indoplanorbis exustus	...	...	870
Macrochlamys sp.	...	...	56
Pila spp. ...	...	...	15
Segmentina spp.	...	...	10
Vivipara bengalensis	...	...	71

All the above species of snails and also *melanoides* were exposed to the *miracidia* throughout the period of the enquiry.

The edible water-plants in the shallow water which are eaten raw in the locality and the water itself where children waddle and catch fish, in the vicinity of houses which harbour known cases of *G. hominis* infestation were examined on a large scale for *metacercariæ*. No *distome metacercariæ* were found. A few *cyclops* were also exposed to the *miracidia* without any result. As the grant of the enquiry terminates this year as recommended by the Clinical Advisory Committee, it is not proposed to continue it.

The lack of the knowledge of the life-history of *Gastrodiscoides hominis* leaves a gap in the literature of medical helminthology and further work on the subject is indicated. The problem can only be solved by a staff stationed permanently in the endemic area in Assam.

11. Enquiry to investigate allergic diseases and significance of endogeneous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

Recent literature reveals several factors influencing the production of allergic conditions. Of them bacterial infections are the foremost. The exact mechanism of bacterial infection in the pathogenesis is, however, unknown. It is not known whether it perverts the function of the vital organs and endocrines and makes the organisms susceptible to subsequent sensitization by metabolic toxic products (allergens), or it is a synergistic combination between tissue-proteins and bacterial toxins giving rise to auto-antibodies noxious to the organisms. Direct approach to the problem is difficult, as we do not know where to start.

In investigating, at this Laboratory, the ætiology and diagnosis of endogenous cases it has been tried to utilize Urback's contention that urinary proteose is an endogenous allergen. Auto-uro-proteose-therapy has been tried on 27 cases with, undoubtedly, encouraging results. Skin tests with auto-proteose solutions have been tried on all cases and none showed negative response. Most revealing fact in skin-tests observed in this series is a definite relation between the symptom-complex and the test. Some cases responded with remark-

able results showing complete amelioration of clinical symptoms while others showed aggravation. Doses of the therapy were determined by the sensitivity of the patient to his own proteose as determined by skin tests. Technique of extraction of proteose and procedure for skin-testing and auto-proteose therapy were the same as reported in last year's note. The results of skin-testing and therapy on this series of 27 cases are given in the chart :—

Results of auto-proteose therapy.

Disease.	Number of cases.	Positive skin-test.	Therapeutic results.			
			Total.	75 per cent.	50 per cent.	*Nil.*
Asthma	18	18	5	8	1	4
Dermatitis ...	5	5	3	1	...	1
Urticaria	1	1	...	...	1	...
Rhinitis	1	1	...	1	...	...
Psoriasis	1	1	...	1	...	...
Rheumatoid arthritis ...	1	1	Improving	...	...	...
Total ...	27	27	8	11 (1 Improving)	2	5

Antibody demonstration.—To explain scientifically these apparent effects of auto-proteose therapy—whether a specific or hetero-specific hypo-sensitization (*cf.* peptone therapy)—attempts are being made to ascertain the allergic nature of proteose. The allergen, being antigen, gives rise theoretically to specific antibodies in the system of the best. Oridnary immunological techniques employed in the laboratory have been occasionally tried to demonstrate these allergic antibodies. So far only a few regains against oxogenous allergens have been demonstrated. Urbach by modern technique of Cannon and Marshall demonstrated specific precipitins in the sera from patients known to be hypersensitive to egg-protein, tuberculin and crystalline insulin. Györgyi *et al.* demonstrated complement-fixing antibodies to egg-white in hyper-sensitive children, while Jaffe and Urbach to pollens, fish, yeast and lentils. Endogenous allergic antibodies have not, however, yet been identified as the exact nature of the allergen to be employed in the test is unknown. With the hypothesis that urinary proteose is related to endogenous allergic state complement-fixation tests were employed in demonstrating antibodies to urinary proteose. So far 11 allergic patients' blood was tested with auto-proteose as antigen and positive results were obtained in 7 cases. Taking tuberculosis to be essentially an allergic disease, the same technique was applied for detecting anti-proteose antibodies in tubercular patients' blood. Up till now 40 unselected cases were rested, 30 of them showed definite inhibition of hæmolysis of sensitized r.b.c. The antigen in the test was urinary proteose extracted from urine and emulsified with Evan's solution to opacity tube No. 10. Serum tested was inactivated at 55°C. for half an hour. The working dilution of the serum was 1 in 5. The working dilution of the antigen was determined by anti-complementary property. It was usually 1 in 4. Complement was used in 2 m.h.d. (original titre not less than 1 in 40). The hæmolytic system was 3 per cent suspension of sheep's r.b.c. with 5 m.h.d. ambocepter. The results of the test are as follows :—

Complement-fixation test with urinary proteose as antigen.

Antigen used in the tests.	Nature of cases.	Number of cases.	Results.		
			Positive.	Doubtful.	Negative.
Auto-proteose	Allergic cases ...	11	7	*Nil.*	4
Tubercular proteose	Tubercular allergy ...	40	30	*Nil.*	10
	Non-tubercular allergy	4	*Nil.*	*Nil.*	4
	Normal cases ...	3	*Nil.*	*Nil.*	3
Hetero-proteose	Tubercular allergy	5	*Nil.*	*Nil.*	5
	Non-tubercular allergy	5	*Nil.*	1	4
	Normal cases ...	3	*Nil.*	*Nil.*	3

Antigenicity in animals.—Both rabbits and guinea-pigs were injected with proteose emulsions as used in complement-fixation tests. Doses in rabbits were 0·25 c.c., 0·5 c.c., 1 c.c., 2 c.c., 3 c.c., and 4 c.c. at weekly intervals intravenously, while the doses in guinea-pigs were 0·25 c.c., 0·5 c.c., 1 c.c. intracardiac (weekly). Blood was tested one week after the last injection for antibodies by complement-fixation tests. Technique was the same as described above. The titre of antibodies was fairly high in practically each case.

Anti-proteose antibodies in rabbits and guinea-pigs.

Proteose number.	Antibodies in :	
	Rabbits.	Guinea-pigs.
7	Positive.	Positive.
11 (T)	,,	,,
22	,,	,,
24	,,	Not done.
28	,,	,, ,,

Anti-proteose antibody titres in guinea-pigs.

Animal number.	Antigen.	1 in.						
		10	20	50	100	250	500	1000
1	7	+	+	+	+	+	?	–
2	11 (T)	+	+	+	+	?	–	–
3	22	+	+	+	+	–	–	–

Anaphylaxis in animals.—Rabbit No. 3 tested for anti-proteose antibodies to proteose No. 7 was injected 0·5 c.c. of same proteose after 8 weeks. The animal died of anaphylactic shock within 8 hours. Liver showed extensive damage, lungs acute collapse, kidney patchy degeneration and heart angiometous dilatations of capilleries.

Specificity of proteose.—The anti-proteose antibodies in animals seem to be specific to auto-proteose and give no cross reactions with different samples (25 such) of proteose extracted from different specimens of urine. To scrutinize the rôle of bacteria in determining this specificity, anti-proteose antibodies were tested with different bacterial antigens. The results are denoted below :—

Results of complement-fixation tests with anti-proteose sera (guinea-pigs) and various antigens.

Antigens injected into guinea-pigs.	Complement-fixation test with anti-sera and various antigens.											
	P_1	P_2	P_3	P_4	P_5	1	2	3	4	5	6	7
Proteose (P_1) from a case of neuro-dermatitis (*Streptococcal* allergy)	P	N	N	N	N	P	N	N	N	N	N	N
Proteose (P_2) from a case of tuberculosis (pulmonary) ...	N	P	N	N	N	N	P	N	N	N	N	N
Proteose (P_3) from a case of psoriasis	N	N	P	N	N	N	N	N	N	N	N	N
Proteose (P_4) from a case of pleurisy	N	?	N	P	N	N	P	N	P	N	N	N
Proteose (P_5) from a case of ? abdominal tuberculosis ...	N	P	N	N	P	N	P	N	N	N	N	N

1 means *Streptococcal* antigen (throat).
2 means tubercular antigen.
3 means *Mic. catarrhalis.*
4 means *Staphylo-albus* (throat).
5 means *Staphylococcus albus* (throat).
6 means *B. coli* (stool).
7 means *Salmonella.*

P indicates presence of inhibition of hæmolysis.

N indicates no inhibition of hæmolysis.

Specificity of proteose as evidenced in our observations may be due to haptonization of bacterial toxins by tissue-proteins. These facts may establish an important theory relating to the rôle of bacterial infection in the pathogenisis of allergic state.

From the foregoing observations the following conclusions may be drawn :—

(i) Auto-proteose may be effectively used in some of allergic cases (endogenous) as a therapeutic agent.

(ii) Auto-proteose seems to be specific and specificity is due probably to incorporation in it of bacterial hapten to which the patient is sensitive.

(iii) Urinary proteose in tubercular patient seems to contain tubercular hapten.

These facts may, therefore, open up important lines of research as they may be utilized :—

(i) In investigating the causative factor of conditions of unknown aetiology.

(ii) Furnishing us with the antigen of different pathogenic organisms hitherto unapproachable (e.g. malaria, filaria, etc.) for performing and studying antigen-antibody reaction *in vitro*.

(iii) In diagnosing the tubercular condition in active phase of the disease, even in early stage where clinical methods may not reveal the presence of specific infection.

The future line of work has, therefore, been planned for elucidation of these problems.

12. Enquiry on the enteric group of fevers under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

1. Hospital cases.

One hundred and eighty five cases (admitted into the Carmichael Hospital for Tropical Diseases, Medical College Hospitals and Campbell Hospital) were studied of which 72 were bacteriologically positive (*B. typhosum* 64 and *B. paratyphosum* A 8). The remaining 113 cases were diagnosed only by Widal reaction with the rising of titre. The latter group consisted of typhoid 89, paratyphoid A 21, paratyphoid B 2 and *B. enteritides* infection 1. No case of paratyphoid C was encountered. Thus, the army report that there has been a marked increase of *Salmonella* infection in recent years cannot be substantiated among the civil hospital population in Calcutta.

Though cases generally occurred at all times in the year, the largest admission was noticed during the months of June and July. Of the 185 admissions, 108 were males and 77 females; the hospital accommodation for females was, however, limited. Forty per cent of the cases were between 11 and 20 years and a little over 30 per cent of the cases were between 21 and 30 years of age, the youngest one being 4 and the oldest 62 years of age. Except one all were Indians.

Out of the 185 cases 60 were critically examined and the findings were recorded in a specially prepared *pro forma*. Among them 31 were bacteriologically positive (*B. typhosum* in 27 and *B. paratyphosum* A in 4 cases) and in 29 the diagnosis was made from the Widal reaction alone (typhoid 27, paratyphoid A 1 and *B. enteritidis* infection 1). Four patients had inoculation within six months to 2 years prior to the attack. In 12 cases the infection was presumably contracted from other typhoid patients in the family. Headache and mild or severe tympanitis were common symptoms. The former was most common during the first week and the latter during the second and third week. Certain atypical symptoms were noticed during the course of the disease such as repeated chills, frequent vomiting, epistaxis, excessive sweating, clear and moist tongue, dysuria and retention of urine, intermitant and irregular temperatures. Nervous symptoms were uncommon in the first week. Typical typhoid rash was noticed on the chest and abdomen in three cases about the 10th day and petechial rash in 3 cases about the third week. Bradycardia with a pulse-rate of less than 100 per minute was met within 3 cases

during the first week and in 2 during the second week. Five patients had irregular pulse, one had auricular fibrillation, and 4 had extra-systole. Weakness of the first heart sound as common. Mitral systolic murmur was audible in 7 cases between the second and fourth weeks and in all of them the sound, however, returned to normal during convalescence. Either bronchitis or basal congestion was pronounced in 17 cases during the second, in 18 during the third and in 2 cases during the fourth week. Thirty-five patients were markedly ill and the most severe cases were generally those who came into the hospital at a late stage of the disease and whose diet had been greatly restricted. Complications such as broncho-pneumonia was noticed in 9 cases, circulatory failure in 6, hæmorrhage in 6, jaundice in 1, parotitis in 2, 'tyhpoid spine' in 1, and abscess in 3. The notable feature about the last 3 cases was the failure to detect typhoid bacilli in the pus. Five patients had relapsed (8·5 per cent).

There had been 27 deaths amongst 185 cases, all infected with *B. typhosum*. The mortality amongst those who were given adequate diet in the hospital was 3 out of 72 cases (4·1 per cent). In other cases where the diet had been restricted the death-rate was 24 out of 113 cases (21·1 per cent). Causes of death were hæmorrhage in 14, circulatory failure in 12 and pneumonia in 1.

2. Variation of plasma-protein levels during the course of the disease.

Plasma-proteins were estimated on 72 occasions in 35 patients. It was found that marked diminution of plasma-proteins at the expense of albumin fraction occurred during the whole febrile course of the disease but the plamsa-protein pattern tended to return to normalcy during convalescence. Mean plasma-protein concentrations were as follows:—

Week of the disease.	Number of observations.	Plasma-protein (g. per cent):		
		Total.	Albumin.	Globulin.
1st ...	4	5·6	3·0	2·6
2nd ...	16	5·4	2·8	2·6
3rd ...	21	5·5	2·6	2·9
4th ...	15	5·6	2·5	3·1
5th ...	16	6·7	3·4	3·3
	Normal ...	7·2	4·5	2·7

Hypoprotenæmia and hypoalbuminæmia appeared as early as the first week and became more marked during the fastigium of illness. This was usually pronounced when the toxæmia was marked and/or associated with severe secondary infection. This condition was noticed to be further aggravated if the diet was restricted, but did not improve when the quantity of protein in the diet was increased. With recovery, however, the normal level was noticed to be restored.

The depletion of protein may actually be more serious than it appears, for in the toxic and dehydrated patients there is likely to be considerable diminution of plasma-volume with a great reduction in the actual amount of circulating protein. Only estimation of blood volume along with plasma-protein determination can clarify this point.

3. Glucose-absorption and glucose-tolerance.

With a view to find out the absorption of glucose in different conditions of this disease glucose-tolerance test was carried out in a series of 27 cases during the height of the illness. It was found that glucose by mouth was readily absorbed from the gut. In 10 cases the curve was normal but in the rest it was abnormally high indicating impairment of glucose-tolerance. In 8 cases the curve was of a diabetic type and in 4 sugar was detected in the urine during the test. During the convalescence period the glucose-tolerance test was normal. Similar findings were recorded in starvation cases during Bengal Famine.

4. Diet.

To counteract the excessive protein destruction which takes place in this disease specially when the diet has been grossly restricted, suitable diet scales to suit different conditions have been prepared. The details have been submitted for publication in the *Indian Journal of Medical Association*. However, the basic principles adopted are given below :—

Total calories prescribed, about 40 calories per kg. body-weight, varied in this series from 2,000 to 3,000, the actual amount being determined by the condition of the individual. The amount of protein given, varied from 60 g. to 90 g. (about 1 g. to 1½ g. per kg.), and that of fat, was almost the same as protein. The bulk of the food was made up of carbohydrate (about 300 g. to 400 g.). A pertinent question arises whether it is advisable to give so much carbohydrate in view of their defective utilization as revealed by glucose-tolerance test. It has been observed that when feeds containing the above quantity are given at regular intervals, sugar does not appear in the urine.

Feeds were given every 2 hours in most of the cases and ample fluid intake was ensured, about five pints a day. The patients with proper feedings in the above line looked well presenting a clinical picture quite different from the classical text-book description and the mortality rate was low.

5. Hypoproteinæmia and prognosis.

The degree of hyproproteinaemia noticed during the course of the illness had a definite correlationship with the severity of the illness and the prognosis. In seriously ill patients the total plasma protein varied from 5·1 g. to 5·5 g. per cent on an average and the albumin fraction from 2·4 g. to 2·7 g. per cent, while the corresponding figure in milder cases were 5·6 g. to 6·2 g. per cent and 2·8 g. to 3·0 g. per cent.

Cannon (1942-43) postulated from studies on laboratory animals that decreased protein-reserve brought about by prolonged low-protein diet leads to the decrease in the production of specific antibodies which are nothing but specially modified globulins. This hypothesis was tested on 31 cases and from 56 observations of blood-protein and agglutination reaction it became apparent that no such correlationship could be established in typhoid cases where hypoproteinæmia is common. There were cases where the total protein was low and the globulin concentration was normal but a rise in the titre of agglutination was all the more present. From the observations of Cannon it was thought that the reduction of plasma-protein in typhoid may have adverse effect on the production of antibodies but this has not been fully substantiated by the

present study. Incidentally it may be mentioned that T.A.B. inoculation was given to a number of patients showing marked signs of malnutrition accompanied by hypoproteinæmia and nearly all of them had a good immunological response. The reason of the rise in the titre of typhoid should be looked at from a different angle and not solely connected with hypoproteinæmia.

6. Treatment.

(a) *Polyperin.*—Though the number of cases in which this antibiotic was tried was small, no appreciable result was noticed. As the preparation of the product has now been discontinued further trials are not possible.

(b) *Penicillin and sulphathiazole.*—Synergic action of penicillin and sulphathiazole has been studied in 13 cases only (8 males and 5 females). Among them 12 were bacteriologically positive. The treatment was started from the 9th to 13th day of illness. Nine of them were seriously ill. 200,000 units of penicillin were given every 2 hours and sulphathiazole 1 g. every 3 hours. The first dose of sulphathiazole 1 g. was given intravenously. The course lasted for 5 to 7 days. The temperature became normal in 6 to 18 days after the treatment was started. One patient died during the course of the treatment. A definite improvement was noticed in patients who were severely ill and who had marked pulmonary complications, but it cannot be stated that this treatment had the desired effect in all the cases on reduction of temperature and lessening of toxæmia.

13. Enquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay.

For this study lens and uveal allergens are required. The study has been confined to only one of them, at present the lens, because from our preliminary study we have found it to be far more important than the uveal.

The lens allergen was prepared by a slight modification of the method described for its preparation by Woods and Burky. Briefly speaking 35 lenses were taken from the eyes of freshly slaughtered buffaloes and put in a sterile flask containing broken glass and 250 c.c. of N/100 ammonium-hydroxide solution. This was left in a refrigerator for eight days. Then the flask was shaken for 24 hours in a mechanical shaker improvized from an old duplicating machine. Then 400 c.c. of N/100 ammonium-hydroxide solution were added so that for every lens there were 20 c.c. of the solvent. The fluid was centrifuged twice and the supernatant fluid was passed through Seitz filter. This had to be done thrice till sterility tests showed no growth on blood-agar and glucose-broth. Necessary amount of water and 8·5 per cent solution of sodium chloride were added to make a 1 per cent solution of the lens allergen in 0·85 per cent sodium-chloride solution. Similarly, an extract was made of pigs' lenses and human cataractous lenses.

This method of preparation is very slow and these allergens were not ready till the end of August, and so no clinical investigations could be carried out with these preparations. However, during this period the clinical investigation on lens-protein sensitivity was started on 1st July with some lens-protein which was previously prepared for our preliminary study. During July and August 91 cases have been investigated. In each the cutaneous sensitivity of the patient to the lens extract was tested before and after the operation. This was done with a view to find out :—

1. Whether the occurrence of phaco-anaphylactic iritis coincided with the discovery of any focus of infection or not.

2. How long after the operation the iritis sets in ?

3. How long after the operation the dermal sensitivity develops, so that one may know whether dermal sensitivity runs parallel with ocular hypersensitivity and whether dermal sensitivity may be taken as a guide to ocular sensitivity in investigations on human beings ?

14. Enquiry on the neurogenic factor in the production of traumatic shock and its treatment by alteration of cerebrospinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

A series of seven animal experiments on dogs have been done up till now. The shock was produced in dogs by various means and the effect on acartoid blood pressure noted after intra-cisternal injection of the solution prepared. A definite rise in blood-pressure is noted which lasted for 10 to 15 minutes.

The necessary solution of potassium mono- and di-hydrogen phosphates has been prepared, ampouled and sterilized and is now ready for clinical trial.

The clinical ward is ready but owing to staff difficulties cannot start functioning before 1st of October when clinical trials are expected to be started.

So far the results in animal experiments are encouraging.

15. Enquiry on the erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval, Serologist to the Government of India, School of Tropical Medicine, Calcutta.

The objects of the enquiry are :—

(a) To detect the development of the Rh antibody in the expectant mothers.

(b) To advise treatment of such mothers, checking results serologically.

(c) To collect human Rh testing sera.

(d) To improve animal testing sera.

(1) Three iso-immunized mothers were detected in a quick succession.

(2) Two anti-Rh sera, type Rh_1 (the commonest type of anti-Rh sera) and anti-Rh (anti-Rh) each of a sufficiently high titre to be used as a testing serum in subjects O were collected for the first time.

(3) Out of 65 unselected bloods (examined in connection with item 1) 5 were found to be Rh-negative. The findings under (1) and (3) are not in conformity with a negligible Rh-negative rate in Indian.

16. Enquiry to investigate bactericidal, protistocidal and fungicidal action of phytoncides under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

Plants have been known for long to be useful in the treatment of infectious diseases. But no systematic investigations on the existence of antibacterial substance in plants, their isolation and activity, *in vitro* and *in vivo*, has been taken up by the scientific world until recently. A systematic investigation has

been done by Osborn (*Brit. Jour. Exper. Path.*, 1943, **24**, pp. 227-31), on the antibacterial activity of 166 families consisting of 2,300 plants. Cavallito *et al.* (*Jour. Amer. Chem. Soc.*, 1944, **66**, pp. 1950-51 ; *Ibid*, 1946, **68**, 2332) have tried to isolate the active principles of several plants and studied their antibacterial activity *in vitro*. Russian workers like Tokin, Filatova and Toropzev *et al.* (Microbiology and Epidiomology ', Chap. XIII, ' Phytoncides ' by B. Tokin) studied 200 plants for their antibacterial nature. Of all the plants studied the garlic and the onion have been claimed by Russian workers to be the most active in killing bacteria, protozoa and pathogenic fungi. They claimed that plants can synthetize during the course of their metabolism substances of unknown chemical nature, which protect them against bacterial and fungal invasion. To such substances Tokin (1944, *Amer. Rev. Soviet. Med.*, **1**, p. 237) attributed the name of phytoncides.

Since garlic has been claimed to contain the most strongly active antibacterial substance this enquiry was taken up to isolate the active principle from garlic (*Allium sativum*) and to study its antibacterial activity. The garlic antibacterial, named by Cavallito (*Jour. Amer. Chem. Soc.*, 1944, **66**, pp. 1950-51) as allicin, has been found to be an ether soluble unstable oily substance, slightly soluble in water, having a molecular formula $C_3H_5SOSC_5H_5$ and is enzymically liberated from an inactive precursor in garlic (*Jour. Amer. Chem. Soc.*, 1945, **67**, pp. 1032-33). Tokin and other Russian workers have worked only with the fumes of garlic and onion, which is bactericidal and protistocidal in nature.

The work of Tokin was repeated by us as far as practicable and it was found that the volatile emanations of garlic possess antibacterial activity against *Streptococcus hæmolyticus*, and *Staphylococcus aureus* but not against *B. coli* and *B. typhosum*.

Extractions of garlic cloves with solvents like alcohol, ether, chloroform, acetone, petroleum ether were tried. Only in the ether and chloroform soluble fractions activity against *S. aureus* and *B. coli* was found. The activity of different extractives is summarized in Table I :—

TABLE I.

Fractions of garlic.	Bacteria used.	Results.	Concentration.
1. Alcohol-soluble fraction	*B. coli*	Growth	1 in 1,000
	S. aureus	,,	
2. Chloroform-soluble fraction ...	*B. coli*	No growth	
	S. aureus	,,	
3. Acetone-soluble fraction ...	*B. coli*	,,	
	S. aureus	Growth	
4. Ether-soluble fraction ...	*B. coli*	No growth	
	S. aureus	,,	
5. Petroleum-ether-soluble fraction	*B. coli*	Growth	
	S. aureus	,,	

The protein present in garlic was also tested for antibacterial activity against *S. aureus* and *B. coli*. It, however, showed no inhibition at a concentration of 1 in 1,000.

With the above preliminary observations the following method was developed for the preparation of the active principle. It was found to yield a product sufficiently active :—

One kg. of garlic cloves after removal of the root hairs and dry skin is cooled to 0°C in ice-box for 2 to 3 hours. The cloves are then thoroughly crushed in a previously cooled mincer and at once laked with 2 litres of cold (5°C) distilled water. The whole extract is kept overnight in a frigidaire. The solution is filtered by means of a lint cloth and the juice is pressed out of the cells. The aqueous extract is slightly turbid. This is adjusted to pH 3·8 with 10 per cent sulphuric acid and the proteins are precipitated. The supernatant fluid is centrifuged and the centrifugate kept in frigidaire. The proteins are washed with 200 c.c. protein of distilled water and again centrifuged. The wash is added to the original supernate. The pH of the solution is raised to 6. The total aqueous extract (2·5 litres) is concentrated under reduced pressure and low temperature to half its volume. The concentrated solution is then extracted twice with 200 c.c. portions of ethyl ether and the ethereal layer containing the active substance is kept in the frigidaire for further work. It is treated with anhydrous Na_2SO_4 to remove moisture. The ether is removed at a reduced pressure of 15 mm. to 20 mm. The residue left after removal of ether is treated with 250 c.c. of cold water whereby an emulsion is obtained. The emulsion is treated with 10 c.c. of n hexane to remove the yellow pigments and other hexane-soluble substances. The aqueous extract is separated and again shaken with twice 50 c.c. portions of ether. The ether is again concentrated at reduced pressure (10 mm. to 15 mm.). The residue is a colourless oily liquid which solidifies on keeping, and this is found to be the active antibacterial substance of garlic. The yield is 0·5 g. per kg. The substance is dissolved in sterile re-distilled water, centrifuged and the clear centrifugate is kept. The solution was diluted to contain 0·1 mg. /c.c. and kept in frigidaire for bacteriological investigation.

The test of antibacterial activity was carried out by following the serial dilution method. The broth used contained 1 per cent glucose, 1 per cent peptone, 0·5 per cent sodium chloride and meat extract, adjusted to pH 7. Five c.c. of sterile broth was pipetted aseptically into sterile tubes. One loopful of bacterial emulsion of test organism in normal saline (1,000 million/c.c.) was the inoculum used in the 5 c.c. broth. Phytoncide extract was added to the culture tubes to maintain concentrations of 1 in 50,000 1 in 100,000 and 1 in 150,000. A control tube containing the test organism without phytoncide extract was run in each case. The culture tubes were incubated for 18 hours at 37°C. The growth was observed after the incubation period, the end-point of inhibition was sharp, the effective concentration showing no growth, the next in the series showing good growth. The concentration showing no growth was tested for bactericidal or bacteriostatic action by removing one loopful of culture solution into sterile 5 c.c. glucose-peptone meat-extract broth. Re-inoculation showed no growth showing bactericidal nature of the garlic phytencide. Other workers claimed bacteriostatic nature of the garlic phytoncide, but our results indicate bactericidal nature. Table II summarizes the results obtained :—

TABLE II.

Organism studied.	Concentrations of garlic phytoncide in broth.		
	1 in 50,000	1 in 100,000	1 in 150,000
Staphylococcus aureus ...	No growth	No growth	Growth
Streptococcus hæmolyticus ...			
B. coli			
B. typhosum			
V. choleræ			
B. paratyphosum A ...			
B. paratyphosum B ...			
B. dysenteriæ shiga ...			
B. dysenteriæ Flexner ...			
B. proteus			

A study of the nature of the garlic phytoncide, as to how it exists in the cells of garlic was made. The enzymic liberation of garlic phytoncide, from its inactive precursor was experimented upon by following the technique of Cavallito (*Jour. Amer. Chem. Soc.*, 1945, **67**, pp. 1032-33,) with slight modifications. Acetone extraction of crushed garlic cloves cooled with freezing mixture, was made and the cloves dried at 70°C. When the dried garlic cloves were extracted in the usual manner by treatment with water, a very weak active ether-soluble fraction was obtained. This portion of the work is in a preliminary stage and further work is being done on this line, as to determine whether the phytoncide exists as a free substance, or as a bound precurser from which the active compound is liberated only after enzymic action.

The effect of variation of pH on garlic phytoncide has been studied. The phytoncide solution is fairly stable up to a pH of 3. Effect of alkaline pH is being studied.

The toxicity of the extract of garlic phytoncide was tested on guinea-pigs. Five c.c. of the garlic phytoncide extract (0·1 mg./c.c.) have been injected intra-cardiac into guinea-pigs (body-weight between 300 g. and 400 g.) at intervals of 3 days during a period of 3 weeks. The extract was found to exert no harmful effect on the body-weight, growth and appetite of the guinea-pigs. Experiments to determine the lethal dose are in progress. A study of the effect of the garlic phytoncide on experimental cholera and tuberculosis (*in vivo* and *in vitro*) and on protozoal cells and pathogenic fungi *in vitro* is also in progress.

The above work shows that the phytoncide present in garlic is a fairly active antibacterial substance and plants possess antibiotic substances, a study of whose isolation and activity opens before us a field of fruitful investigation.

17. Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji, Director Central Drugs Laboratory, Calcutta.

I. Comparative study of the different methods of extraction of the melanophore hormone of beef pituitary gland.

Efforts were made to extract the melanophore hormone of the fresh beef pituitary gland secured from local slaughter-houses and brought to the laboratory in iced containers, and thermos flask, following in the initial stages

the methods already laid down by previous investigators. It has been found that an acetic acid extract of the fresh whole pituitary gland is highly potent in melanophore activity, this being determined by assay methods to be described later. Dilute pyridine extract of pituitary prepared by the method described by Abrahmowitz *et al.* (*Jour. Biol. Chem.*, 1943, **151**, p. 579) shows about 50 per cent activity. Extraction of the hormone by dilute ammonia is found to be unsatisfactory. Extraction of melanophore hormone from the acetone-dried whole pituitary gland was found to give on an average 50 per cent activity. Hormone extracted and purified by the method of Stehle (*Jour. Pharmacol. Exper. Therap.*, 1936, **57**, p. 1) indicated also an activity in the range between 45 and 50 per cent.

In order to concentrate and purify the hormone, a systematic investigation to study the following physico-chemical conditions have been undertaken :—

(a) *The effect of pH.*—It has been observed that melanophore activity is destroyed to the extent of about 20 per cent when pH of the extract is lowered to 2·0. Similar diminution of activity, but to a lesser extent, was observed when the pH of the solution was raised to 8·0. The optimum activity remains at pH 4·0.

(b) *The effect of heat.*—Melanophore hormone is apparently heat-stable though it cannot stand prolonged heat treatment even at as low a temperature as 40°C. Complete destruction of activity was observed when acetic acid extract of the pituitary was subjected to slow evaporation under hood at 40°C. Vacuum evaporation under continued suction by pump at a temperature below 50°C. caused slight but appreciable destruction of hormone activity. Addition of sufficient absolute alcohol to the acetic acid extract removed inert materials and the alcoholic filtrate on vaccum evaporation at a temperature below 50°C. retained almost the same hormone activity.

(c) *The effect of adsorbing agents.*—Concentration of melanophore hormone by adsorption on suitable adsorbing materials such as 'activated carbon' and 'Decalso' has so far been attempted. Active carbon is found to adsorb completely the melanophore hormone, while 'Decalso' is partially effective. Further study made with other adsorbing agents like Fuller's earth, kaolin, calcium phosphate, lead acetate, alumina gel, silica gel, zeolite, bentonite, etc., is being planned. Once the optimum conditions for adsorbtion is defined, attempts will be made to elude out the hormone quantitatively from the adsorbate. This will be taken up before very long.

(d) *Effect of precipitating agents.*—So far, two precipitating agents such as acetone and ammonium sulphate have been tried on the acetic acid extract of the pituitary gland. Sufficient quantity (1 : 4 by volume) of acetone is capable of producing precipitates which contained melanophore activity. The acetone precipitate is found to be 4·5 times as active as fresh tissue.

Half saturation with ammonium sulphate is effective in almost completely removing hormone activity from the solution. The precipitate containing sufficient amount of ammonium salts possessed hormone activity. Removal of ammonium salt by dialysis inside a cellophone chamber has been carried out. Preliminary investigation indicates that a small but appreciable amount of melanophore hormone is dialysed out of the cellophone chamber during 24 hours, though the major portion of the hormone remains inside the chamber.

A plan has been made to concentrate the hormone further by precipitation of the dialysate with requisite quantity of acetone. If required, the ammonium-

sulphate precipitate may be subjected to electrodialysis. From this stage onward, each lot in the process of concentration will have to be assayed for the presence of other associated pituitary hormones, e.g. oxytocic and pressor hormones (including the anti-diuretic factor) of the posterior pituitary lobe and the other anterior pituitary hormones. The determinations of nitrogen by Kjeldehl method and also of amoni-acids like tyrosine, cysteine, etc., chemically and by paper chromatography, if necessary. When sufficient concentration of the melanophore hormone will be effected, and the material thus obtained will, on assay, be found to be reasonably stable and non-toxic on experimental animals, clinical trials will be taken up.

(e) *Stability.*—The melanophore hormone in acetic-acid solution is found to deteriorate slowly. It has been observed that the acetic-acid extract lost about 50 per cent of its activity when kept in a refrigerator (8°C. to 10°C.). Attempts will be made to improve the stability of the product by the addition of suitable anti-oxidants, e.g. hydroquinone, ascorbic acid (vitamin C), sodium sulphate, etc.

II. Study of the pharmacological methods of assay and standardization of the melanophore hormone.

(a) *Assay on living frogs.*—Until recently the melanophore hormone was assayed by measuring the degree of darkening of the skin of pale *Rana* (frog) or other amphibia injected with the hormone. When a definite quantity of hormone preparation is injected into a lymph sac of a pale *Rana*, the darkening of the skin develops almost instantaneously. The darkening of the skin is due to the expansion of the melanophore cells under the influence of the pituitary hormone. By injecting small doses of a hormone preparation, it is possible to obtain a ' minimum dose ' which is just sufficient to develop a visible darkening in a pale *Rana*. This dose is known as ' minimum dose response '. Thus by comparing the minimum dose response of a ' standard ' with that of an unkown preparation, it is possible to assay melanophore activity. It is known that the sensitiveness of this dose-response ratio can be significantly improved in hypophysectomized frogs. This procedure will be attempted later.

(b) *Counting of melanophores.*—Landgrebe and Waring (*Quart. Jour. Exper. Physiol.*, 1946, **33**, p. 1) have described a quantitative method for the assay of the melanophore hormone by counting microscopically the number of dermal melanophores in a particular web (generally the central web) in the skin of the *Xenopus*. Similar procedure is applicable to the *Rana* but the skin of *Rana* has a large number of epidermal melanophores in addition to the normal dermal melanophores. These epidermal melanophores tend to vitiate ' quantitative ' estimation under the microscope. Moreover, injection of melanophore hormone sometime produces a ' jacketing ' effect, i.e. darkens the adjacent surface inside the web and thereby interferes with the visual observations. This method, therefore, does not appear to give as satisfactory results in frogs as is reported in *Xenopus*.

(c) *Assay on isolated frog skin.*—Recently, Frieman, Fishbein and Hisaw (*Arch. Biochem.*, 1948, **17**, p. 183) have described an *in vitro* method for the assay of melanophore hormone. This *in vitro* method with certain modifications has been adopted for the assay of melanophore hormone in this laboratory. In a ' Lumitron ' photoelectric instrument, the skin of a pale *Rana* is fixed in a specially made bakelite frame and suspended in amphibian Ringer solution

inside the cell of the 'Lumitron' apparatus. The amount of light transmitted through the isolated skin of the *Rana* is recorded by the sensitive galvanometer of the 'Lumitron'. Introduction of hormone inside the cell causes expansion of the melanophores and a darkening of the skin surface results. The cutting off of the transmitted light causes a deflection in the galvanometer reading, which measures, within a certain range, the degree of darkening brought about by the influence of the melanophore hormone. With a known standard of melanophore hormone, it is possible to assay different preparations for melanophore activity. This method of assay is found convenient and at the same time appears quite reliable.

An acetic acid (0·5 per cent) extract of standard dry whole pituitary powder representing 1 mg. of powder per c.c. produces definite deviation in the galvanometer deflection and this deviation is arbitrarily chosen by us as representing 100 per cent activity. Melanophore activity of various preparations has been assayed in this way and it has been found that an acetic acid extract of fresh whole pituitary gland of ox, representing 17 mg. of fresh tissue per c.c. possessed 240 per cent activity.

(d) *Interrelation between in vivo and in vitro methods of assay.*—It has been observed that *in vivo* method of assay is more sensitive than that obtainable from the *in vitro* technique. An extract which failed to record any deviation in the galvanometer deflection in *in vitro* experiment, is found to be capable of darkening the skin of a pale *Rana* when injected *in vivo*. Experiments are in progress to establish a quantitative relation between *in vivo* and *in vitro* methods of assay.

(e) *Assay with fish melanin.*—When fish like 'Singhi' (*Heteropneustis fossilis*) and 'Magur' (*Clarias batrachus*) were kept in white and black backgrounds, it was found that the fish kept in white background became paler in colour than those kept in black background. Further, the injection of melanophore hormone in pale fish produced rapid darkening of the skin. Quantitative estimations of the amounts of melanin present in the skin of the fish under the above experimental conditions are in progress. Preliminary results indicate that it might be possible to evolve a new quantitative method for the assay of melanophore hormone.

List of Papers published.

BANERJEE, S., GHOSH, N. C., and BHATTACHARYA, G.—A Chemical Method of Estimation of Nicotinic Acid in Urine in the Presence of Sugar. (*Jour. Biol. Chem.*, 1948, **172**, p. 495).

CHAUDHURI, R. N.—Diet in Typhoid. *Ind. Jour. Med. Assoc.* (*In press*, 1948).

DAS GUPTA, C. R., and CHATTERJEE, J. B.—Folic Acid in the Treatment of Macrocytic Anaemia in Pregnancy (*Ready for publication*).

Report of the Sixth Meeting of the Clinical Research Advisory Committee held in the Senate House, Madras, on the 30th November, 1948.

MEMBERS PRESENT.

Dr. V. R. Khanolkar. (*Chairman*).
Dr. R. N. Chaudhuri.
Dr. R. N. Cooper.
Dr. C. R. Das Gupta.
Dr. S. W. Hardikar.
Dr. M. G. Kini.
Dr. P. Kutumbiah.
Lieut.-Colonel S. Narain.
Dr. J. C. Ray.
Dr. M. V. Radhakrishna Rao.
Dr. B. B. Yodh.
Dr. J. C. Patel. (*Secretary*)

Dr. A. N. Goyle was not able to attend the meeting.

IN ATTENDANCE.

Dr. S. Banerjee.
Dr. A. Benjamin.
Dr. B. B. Dikshit.
Dr. P. V. Gharpure.
Dr. B. P. Gyani.
Lieut.-Colonel C. K. Lakshmanan.
Dr. T. Lakshminarayana.
Dr. B. Mukerji.
Dr. V. R. Naidu.
Dr. V. N. Patwardhan.
Dr. K. Rajagopal.
Dr. D. Govinda Reddy.
Dr. D. V. S. Reddy.
Dr. D. N. Roy.
Dr. D. M. Satur.
Dr. K. S. Sanjivi.
Dr. S. K. Sundaram.
Dr. R. Viswanathan.
Dr. P. M. Wagle.

Item 1.—The Committee considered the progress of work on enquiries sanctioned during the year 1948-49 :—

(i) Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The Committee approved the work carried out by the unit and recommended its continuation.

(ii) Neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The continuation of the unit was recommended. It is expected that the training of the personnel and the equipment required for

the unit will take about two years and that the post of the Senior Research Officer has been discontinued for lack of a suitable qualified person.

(iii) Enquiry on Naga sore under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong.

The report of work was considered and it was noted that the enquiry would terminate on the 31st December, 1948.

(iv) Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

The continuation of the enquiry was recommended.

The Committee recommended the formation of a Sub-Committee to formulate a programme of research on cirrhosis of the liver and allied disorders consisting of Dr. V. R. Khanolkar, Dr. P. Kutumbiah, Dr. V. R. Naidu, Dr. V. N. Patwardhan, Dr. S. W. Hardikar and Dr. P. N. Wahi. It was also recommended that they should meet for this purpose before June, 1949 and submit a scheme for co-ordinated work.

(v) Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay.

The continuation of this enquiry was not recommended.

(vi) Enquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene and Public Health, Calcutta.

The continuation of the enquiry was recommended.

In view of the satisfactory work of Dr. M. L. Ghosh, M.B., Chemist, it was suggested that an accelerated promotion or a change in the grade of Dr. Ghosh may be favourably considered by the Scientific Advisory Board.

The Committee recommended that a young research worker should be associated with the work of Dr. Krishnan and Dr. Smith. This recommendation was made with a view to continue and promote rickettsial work and allied virus research in the country.

The Committee recommended the formation of a Sub-Committee consisting of Dr. K. V. Krishnan (Convener), Major S. L. Kalra, Dr. V. R. Naidu and Dr. D. W. Soman to plan future research work on typhus in India. It was decided that this Sub-Committee should carry out most of its work by correspondence and meet on a date just prior to the annual meeting of the Clinical Research Advisory Committee.

(vii) Enquiry on the effect of vitamins B and C in diabetes under Dr. S. Banerji at the Presidency College, Calcutta.

The continuation of the enquiry was recommended for one year on condition that the investigation during the next year was limited to :—

A. *Clinical.*—The effect of administration of vitamin C on (i) glucose-tolerance test and (ii) on the urinary excretion of sugar and ascorbic acid by diabetic patients will be studied.

B. *Experimental.*—The effect of injection of adrenal-cortical extract and the removal of adrenal glands on the glucose-tolerance test of normal and scorbutic guinea-pigs will be studied.

(viii) Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

The Committee considered the recommendations made by the Therapeutic Trials Committee regarding the establishment of hæmatological units in different centres in India and recommended the setting up of two hæmatological units—one under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta, and the other under Dr. J. C. Patel at the Seth G. S. Medical College, Bombay. The appointment of an additional research assistant in the scale Rs. 160-10-330 was recommended for the hæmatological unit at Calcutta.

The Committee was of opinion that as the studies of hæmatological problems were assuming greater importance and were recurring every year on an increasing scale all hæmatological work should, as far as possible, be carried out in hæmatological units established under the Indian Research Fund Association. All hæmatological investigations be considered and integrated in carefully designed programme of work prior to the annual meeting of the Clinical Research Advisory Committee.

(ix) Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The report of the work was considered and it was noted that this enquiry would terminate on the 31st March, 1949.

(x) Enquiry on the enteric group of fevers under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

The report of the work was considered and it was noted that this enquiry would terminate on the 31st March, 1949.

(xi) Enquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval at the School of Tropical Medicine, Calcutta.

The continuation of the enquiry was recommended for one year.

The Committee further recommended that collection and distribution of Rh testing sera be undertaken by Government Blood Bank organizations.

(xii) Enquiry on cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis under Dr. G. S. Viswanathan at the Andhra Medical College, Vizagapatam.

The Chairman referred to the untimely death of Dr. T. Bhaskara Menon and as a mark of respect to the memory of the deceased the members stood in silence for two minutes.

The continuation of the enquiry was not recommended. It was suggested that a fresh proposal may be sent to this Committee next year.

(xiii) Enquiry entitled "Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis" under Mr. B. N. Banerji and Dr. V. R. Naidu at the Indian Institute of Science, Bangalore, and Sri Krishnarajendra Hospital, Mysore.

The continuation of the enquiry was recommended.

(xiv) Research on lathyrism in the Central Provinces and Berar.

It was noted that Government of C.P. and Berar have decided not to start this investigation in their province for the next year.

(xv) Enquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

The continuation of the enquiry was recommended for one year.

(xvi) Investigation into the bactericidal, protistocidal and fungicidal action of phytonocides under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

The continuation of the enquiry in its present form was not recommended.

(xvii) Enquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay.

The continuation of the enquiry was recommended for one year. The Committee did not recommend increase in the pay of Mr. B. P. Chakravarti, research assistant on the enquiry.

(xviii) Enquiry on the neurogenic factor in the production of traumatic shock and its treatment by the alteration of cerebrospinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

The continuation of the enquiry was recommended for one year. The Committee did not recommend an additional post of laboratory assistant.

(xix) Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta.

The Committee recommended the continuation of the enquiry for one year with a revised budget of Rs. 20,000.

Item 2.—To consider new proposals.—

(A) The Committee made the following recommendations in regard to new enquiries :—

(i) Enquiry into the state of refractions of Indian children of different communities with special reference to myopia under Dr. G. S. Pendse, 631/27, Sadashiv Peth, Poona 2.

This enquiry was recommended for one year only.

(ii) Study of monilla infection of the skin and adjacent mucous membranes under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

This enquiry was recommended with a revised budget of Rs. 8,875.

(iii) Enquiry on the problem of typing typhoid bacilli by means of Vi bacteriophage and treatment of typhoid fever with type specific Vi 'phage under Dr. R. G. Dhayagude and Dr. D. D. Banker at the Seth G. S. Medical College, Bombay.

This enquiry was recommended with a revised budget of Rs. 2,660 on condition that Dr. D. D. Banker would himself carry out the technical work in connection with the enquiry.

(iv) Enquiry on the associated vaccine for prophylactic immunization against typhoid, paratyphoid cholera, dysentery and tetanus by a single inoculation under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

This enquiry was recommended only if necessary funds were available.

(v) Enquiry on the circulatory changes in severe infection under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

This enquiry was recommended with a revised budget of Rs. 7,295. The Committee further recommended that Dr. R. N. Chaudhuri should carry out the work in collaboration with army workers and should consult Lieut.-Colonel S. Narain in this matter.

(B) The following new enquiries were not recommended in view of financial stringency :—

(i) Studies on the experimental alloxan diabetes under Dr. S. Banerjee, Head of the Department of Physiology, Presidency College, Calcutta.

(ii) Investigation into the biochemical changes in diabetes in the tropics under Dr. J. P. Bose at the School of Tropical Medicine, Calcutta.

(iii) Enquiry on dermatophytosis with special reference to trichophytosis under Dr. N. G. Pandalai, Professor of Bacteriology, Andhra Medical College, Vizagapatam.

(iv) Enquiry on the causation of œdema in kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta.

(v) Enquiry on the bacteriophage typing of V forms of *Bacterium typhosum* present in this country under Dr. S. K. Gupta at the School of Tropical Medicine, Calcutta.

(vi) Enquiry on the incidence of the different types of *C. diphtheria* in cases of diphtheria in Bombay and their correlation with clinical severity under Dr. Y. M. Bhende at the Seth G. S. Medical College, Bombay.

(vii) Enquiry into venereal diseases in Calcutta under Lieut.-Colonel S. D. S. Greval, Serologist and Chemical Examiner to the Government of India, School of Tropical Medicine, Calcutta.

(viii) Research on electro-physiology of unstriated muscle under Captain Inderjit Singh at the Medical College, Agra.

(ix) Enquiry to study the value of para-amino salicylic acid and allied compounds in the treatment of tuberculosis under Dr. K. S. Sanjivi at the Stanley Hospital, Madras.

(x) Research on lathyrism under Dr. H. Jacoby, M.D., Pathologist and Physician, States Hospital, Srinagar, Kashmir.

(xi) Studies on hepatic cirrhosis in Indian adults and infants under Dr. P. N. Wahi at the Medical College, Agra.

(xii) Enquiry into isolation of intestinal pathogens and classification of salmonella under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

Item 3.— *To consider the reports of the meetings of the Therapeutic Trials Committee held in September and November, 1918.*—

The reports of the Therapeutic Trials Committee were adopted and are appended to this report.

Item 4.— The Committee next considered the question of joint investigations under the Indian Research Fund Association carried out in recognised institutes and resolved that in addition to the recommendations regarding joint authorship previously formulated by the Indian Research Fund Association the practice relating to joint investigation should be modified so that whenever a trained investigator is available for carrying out any contemplated investigation the responsibility for the work and its report should lie with that worker.

Report of the First Meeting of the Therapeutic Trials Committee, held on the 6th and 7th September, 1948.

PRESENT.

Dr. M. G. Kini. (*Chairman*).
Lieut.-Colonel Jaswant Singh.
Dr. B. Mukerji.
Dr. J. C. Patel. (*Secretary*).

Dr. C. G. Pandit, Secretray, I.R.F.A. kindly attended the meeting.

Dr. M. G. Kini was requested to take the chair.

It was noted that a questionnaire had been issued to all important hospitals in India asking for their co-operation and help in the conduct of clinical trials of new remedies and that only six hospitals had so far expressed their willingness to undertake this work. Replies from others are awaited. Dr. Patel informed the Committee that the K. E. M. Hospital, Bombay, had also agreed to co-operate in such investigations. It was agreed to issue reminders to such of the hospitals as have not replied to the questionnaire before the 30th September, 1948. The Committee decided that information received from the various hospitals should be tabulated and made available as soon as possible to the members of the Committee.

Agenda item 1.—*To frame rules and regulations for the conduct of clinical trials.*—

Dr. Mukerji informed the Committee that he had since received further information on the *pro formas* which are in use in the U.K. and the U.S.A. The Committee, therefore, requested Dr. Mukerji to submit a note for their consideration incorporating all the points available with a view to frame detailed rules and regulations for the conduct of clinical trials of new therapeutic remedies. The note will be circulated to the members of the Committee for opinion.

Agenda item 2.—*To consider a request from Messrs. Boots Pure Drug (India) Ltd., Bombay, regarding carrying out clinical trials of 'Flavazole' and 'Sulphathiazole'.*—

(a) *Flavazole*.—The Committee agreed that on the basis of the note submitted by the Company (i) it would be advisable to undertake clinical trials with Flavazole (ii) that such trials should be undertaken in two or three hospitals where competent workers are available to undertake the work; and (iii) that in testing the action of Flavazole against anærobic organisms in wounds it would be desirable to associate this unit with the clinical trials of drugs on tetanus.

The Committee was of opinion that Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona, who had done work of a similar nature in the past should, if possible, undertake clinical trials with Flavazole in collaboration with a team consisting of surgeons and bacteriologists to be formed by him for this purpose in his own hospital. The Committee was also of opinion that Dr. R. G. Dhayagude, Dean, Seth G. S. Medical College, Bombay and Dr. B. B. Yodh, Professor of Medicine, Grant Medical College, Bombay, who had done work on anærobic wounds and on tetanus respectively should be requested to form similar groups in their hospitals for such trials.

(b) *Sulphathiazole*.—The Committee was of opinion that as Sulphathiazole was a well-known drug it was not quite clear as to why further trials on this

drug in India were considered necessary by the Company. The Secretary, I.R.F.A. was requested to obtain further information from the Company.

Agenda item 3.—To consider a request from the Oriental Pharmaceutical Industries, Ltd., Bombay regarding carrying out of clinical trials of "Asthmopil".—

The Committee considered the request from the Oriental Pharmaceutical Industries Limited, Bombay, for conducting clinical trials with Asthmopil in the treatment of asthma. The Committee noted that this was not a new drug but a prescription containing well-known drugs at present used in the treatment of asthma. The Committee, therefore, decided that trials of such prescriptions did not fall within its purview.

Agenda item 4.—To consider a request from Shri A. Tozhutaparamban of Cochin State regarding carrying out of clinical trials with sodium hypophosphite in the treatment of threadworms.

In considering the request from Shri A. Tozhutaparamban for carrying out clinical trials with sodium hypophosphite in the treatment of threadworms the Committee decided to refer the application back to the applicant asking him to furnish full evidence on which he had based his claim on the efficacy of the drug.

Agenda item 5.—To consider whether it would be possible to carry out clinical trials with 'V-187' in India on behalf of the Medical Research Council of the U.K.—

The Committee was of opinion that the drug V-187 should be tried out in cases of tetanus. The Committee decided to approach workers in Bombay, Calcutta and Madras with a view to formulate urgently definite proposals for the trial of this drug. In addition the Committee requested Drs. Kini, Mukerji and Patel to establish contacts in their respective areas.

Agenda item 6.—To consider a request from Shri Bhagwati Prasad Kulshrestha, Kulshrestha Ayurvedic Pharmacy, Agra Cantt., regarding carrying out of clinical trials with 'Shresthbati'.

Item 8.—To consider a request from Dr. Hakim Rafi-Ud-Din Khan of Bareilly regarding carrying out of clinical trials with 'Malaria Cure'.

Item 10.—To consider a request from Messrs. King & Hudson, Ltd., Coventry, England, regarding carrying out of clinical trials with 'Sankolan'.

The Committee considered items 6, 8 and 10 on the agenda together since the drugs in question all referred to the treatment of malaria.

The Committee did not accept the drugs submitted by the firms concerned for conducting clinical trials as they were no new drugs but merely prescriptions (*see* item 3 of the agenda).

Agenda item 7.—To consider a request from Shri Subodh Chandra Ghosh, M.Sc., Calcutta regarding carrying out of clinical trials with "Neo-Chin".—

The Committee considered the request from Shri Subodh Chandra Ghosh of Calcutta regarding the carrying out of clinical trials with Neo-Chin in the treatment of malaria and recorded that since Neo-Chin contains a cinchonine salt, a detailed investigation should be undertaken both on monkeys and human cases under the direction of the Malaria Institute of India, Delhi.

genda item 9.—*To consider a request from the Proprietor, Dr. Rao's Laboratory, Bombay, regarding carrying out of clinical trials with liver extracts.*—

In considering the request for clinical trials of liver extract prepared by Dr. Rao in his laboratory in Bombay the Committee was of opinion that there was a pressing need to formulate a definite scheme for clinical trials of liver extracts prepared by various firms in India. A note on the subject prepared by Dr. Mukerji and Dr. Patel was considered by the Committee and adopted. This note is attached as *Appendix* to this report.

The Committee decided to defer consideration of carrying out clinical trials with Dr. Rao's liver extract pending the formation of permanent hæmatological units in different centres in India as envisaged in the note by Drs. Mukerji and Patel.

Agenda item 11.—*Any other business.*—

Dr. Mukerji suggested that the formation of the Therapeutic Trials Committee under the I.R.F.A. should be made widely known in India and abroad. The Committee decided that enough publicity had been given in India regarding the formation of the Therapeutic Trials Committee under the I.R.F.A. and that no further action was necessary. As regards bringing the existence of the Committee to the notice of other countries, the Committee decided to postpone consideration of the proposal to a later date.

APPENDIX.

Note on the Establishment of Hæmatological Units for the Standardization of Liver Extracts manufactured in India.

By

Dr. B. Mukerji and Dr. J. C. Patel.

1. *Present state of the problem in India.*—Nutritional macrocytic anæmias (tropical macrocytic anæmias, macrocytic anæmias of pregnancy and anæmias of sprue and pellagra) are prevalent in India to a greater extent than in any other country of the world. This is due to a number of factors of which the following are the more important :—

(i) Poor economic condition of the majority of the people of this country.
(ii) Lack of balanced diets; and
(iii) Prevalence of tropical diseases like malaria, dysentery, etc., in a large scale.

The only effective method of treatment of these anæmias so far is the administration of liver extracts. The present line of treatment of macrocytic anæmias is administration of liver extracts either orally or parenterally and other drugs like folic acid and methyl thyamine. Of these methods, treatment with liver extracts administered orally is not very effective and comparatively costly. The treatment of anæmias with folic acid is also so costly that it is beyond the reach of the majority of the people of this country and besides this mode of treatment may not be the complete answer to the problem. On the other hand treatment with methyl-thyamine is not considered by a good number of workers as effective as it was claimed to be at one time.

The treatment of anæmias by parenteral administration of liver extract therefore, appears at present to be the only line of choice both from the point of view of its cost and easy availability in India.

Till the outbreak of the second world war, liver extracts used in this country were of foreign origin. These liver extracts are usually standardized for the treatment of cases of Addisonion pernicious anæmias which is largely prevalent in countries having a temperate climate. It is usually mentioned by the manufacturers of these extracts that every single batch of the liver extracts is tested before it is placed on the market. Due to the outbreak of the war, however, owing to the lack of regular supplies from abroad, the manufacture of liver extracts was started and developed extensively in India and there are now several firms in this country which manufacture these extracts which are now to some extent being used by clinicians all over the country. There are, however, no facilities available in India to have these extracts standardized before being put on the market and hence they suffer in competition with those of foreign manufacture. The only basis of standardization available is a statement which the manufacturers furnish, stating that the finished extract is derived from so many grammes of original liver—a statement which is not of much value from the clinical point of view.

2. *What is done in other countries.*—There is no reliable method available at present which will enable these liver extracts to be standardized on laboratory animals. Methods based on increasing the reticulocyte response of suitable laboratory animals have been tried but these cannot be considered as yet to have reached a stage for adoption. Only a nominal control can be exercised on the quality of liver extracts by these methods.

The only methods of standardization of the liver extracts manufactured in his country appears, therefore, to be by clinical trials of these extracts on suitble cases of anæmias. For the effective carrying out of these trials, the ideal ourse would be to set up a central anæmia hospital where anæmia cases from ll over the country could be segregated and studied by hæmatological experts. his procedure is followed in the United States where there are at least three pecialized hospitals in Boston, Ann Arbor (Michigan) and in San Francisco. he liver extracts of the U.S.P. are standardized with the help of the hæmatogical experts in these hospitals in terms of the U.S.P. anti-anæmia units. n Great Britain, facilities exist for special studies of cases of anæmias, though ot on as wide a scale as in the U.S.A. The Medical Research Council and the uffield Unit for Therapeutic Research have facilities in the St. Bartholomew's ospital, London, as well as in the Guy's Hospital for undertaking clinical ials of liver extracts under such eminent experts as Professor L. J. Witts and r. Janet Vaughan. Such facilities do not exist in India and it would also not e possible to have similar facilities such as separate hospitals for anæmias in he present economic conditions prevailing in the country.

. *What is proposed in this country.*—The best course, therefore, would be to et up three or four hæmatological units in different centres where such ork can be carried out effectively. These hæmatological units should be ttached to hospitals where full facilities are available for the study of anæmias uch as those in Bombay, Calcutta and Madras.

Each of these units should have at least ten beds specially allotted to hem and under their entire control with facilities for out-patients investigations, nd should work under the direction of a physician with hæmatological xperience who shall be designated as the Director of the unit. The staff uggested for each of these units should be as under :—

(i)	Assistant Research Officer (medical man) in the scale Rs. 275-25-500-E.B.-30-590	... ...	1
(ii)	Senior Laboratory Assistants in the scale Rs. 60-4-120-5-150	...	2
(iii)	Laboratory Attendants in the scale Rs. 35-1-40-2-60	...	2

These units, in addition to undertaking clinical trials of liver extracts ubmitted by private firms for therapeutic trials to the Indian Research Fund ssociation, will also carry out basic research in the field of hæmatology. The eports submitted by these units will be considered by the Therapeutic Trials ub-Committee of the Clinical Research Advisory Committee at their annual eetings and if considered necessary, the sub-committee may invite the irectors of such units to meet in a joint conference to discuss and exchange iews on the work of these units.

The need for standardization and clinical evaluation of liver extracts anufactured in India is most urgent in the interests of both scientific therapeutics s well as the Indian drug industry. Liver extracts form an important item in he armoury of the modern physician in India and its production on a large ale from the abundant raw materials available in the country would give a llip to the indigenous pharmaceutical industry. The scheme for the estalishment of such hæmatological units on a permanent basis is not likely to nvolve the Indian Research Fund Association in any appreciable extra expenture, since the cost of such trials will to a large extent be borne by the ommercial firms manufacturing these products.

Report of the Second Meeting of the Therapeutic Trials Committee held in Madras on the 23th November, 1948.

PRESENT.

Dr. M. G. Kini. (*Chairman*).
Lieut.-Colonel Jaswant Singh.
Dr. B. Mukerji.
Dr. J. C. Patel. (*Secretary*).

BY INVITATION.

Lieut.-Colonel T. C. Puri.
Lieut.-Colonel S. Narain.
Bt.-Colonel Sir Ram Nath Chopra.

Agenda item 1.—To frame rules and regulations for the conduct of clinical trials of new remedies under the Indian Research Fund Association.—

A draft of rules and regulations for the conduct of clinical trials of new remedies under the Indian Research Fund Association was submitted to the Committee by Dr. Mukerji. After some discussion it was approved with some modifications and is attached as *Appendix I*.

Agenda item 2.—To examine replies received to the questionnaire which was issued to all the hospitals in India asking for their co-operation in the conduct of clinical trials of new therapeutic remedies.—

Out of 160 hospitals to whom the questionnaire was sent, 63 hospitals replied. Out of these, 7 hospitals, viz. Tata Memorial Hospital, Bombay, Seth G. S. Medical College and K. E. M. Hospital, Bombay, J. J. Group of Hospitals, Bombay, B. J. Medical College Hospital, Poona, Government General Hospital, Madras, Government Stanley Hospital, Madras and Gandhi Memorial and Associated Hospitals, Lucknow, were approved for carrying out clinical trials. Replies from other hospitals were awaited. As regards the J. J. Group of Hospitals, Bombay and the B. J. Medical College Hospital, Poona, an assurance was obtained from Dr. Yodh and Dr. Dikshit that these hospitals would co-operate and that they would send replies at an early date.

Agenda item 3.—To consider the scheme submitted through Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona, for the conduct of clinical trials with 'Flavazole'.

Agenda item 4.—To consider the proposals received for the conduct of clinical trials with 'V-187'.—

The Committee requested Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. R. G. Dhavagude, Dean, Seth G. S. Medical College, Bombay to arrange for clinical trials of (i) Flavazole, and (ii) V-187 respectively. The Committee also recommended the necessary expenditure for both the units. The Committee noted the schemes submitted through them from their institutions and agreed that Dr. N. M. Purandare and Dr. N. K. Sahiar, and Drs. B. B. Dikshit, L. B. Joshi and P. G. Gollerkeri be in-charge of these enquiries.

In view of the fact that considerable time had elasped between the request made by the Medical Research Council of the U.K. for clinical trials of V-187 and the arrangements made by the Committee for its clinical trials, the Therapeutic Trials Committee requested the Secretary, Indian Research Fund

Association to ascertain from the Medical Research Council of the U.K. if they still desired the Indian Research Fund Association to carry out clinical trials of V-187.

Agenda item 5.—*To consider a letter from the Director, Haffkine Institute, Bombay regarding the establishment under the auspices of the I.R.F.A. of hæmatological units for the standardization of liver extracts manufactured in India at various centres in India.*—

The Committee discussed the letter of Major-General Sir Sahib Singh Sokhey, Director, Haffkine Institute, Bombay and noted his comments regarding the establishment of hæmatological units. They agreed with him that only manufacturers producing liver extracts in large amount should be encouraged to get their products tested by the hæmatological units under the Indian Research Fund Association. Under the Drugs Act, 1940 and the Rules thereunder the distribution and sale of liver extracts manufactured outside India but sold under Indian label is now prohibited and there would not be any difficulty in the near future of checking such fictitious firms. The Committee also agreed that both oral and parenteral preparations of liver extract should be standardized. The manufacture of liver extract should be encouraged in India. It is true that most of the manufacturers prepare liver extract on a small scale because clinicians have no confidence in a local manufactured product. If their effectiveness is ascertained and potency standardized by clinical trials, the demand will grow and the scale of manufacture of liver extract in this country will increase.

Agenda item 6.—*To consider the proposals received for the establishment of hæmatological units for the standardization of liver extracts manufactured in India.*—

The Committee after going through all the applications for establishment of hæmatological units recommended the setting up of two units one at the School of Tropical Medicine, Calcutta and the other at the Seth G. S. Medical College, Bombay. In addition to the above two units it was recommended that two fellowships in hæmatology be awarded with a view to provide adequate training to two graduates who can later undertake work of hæmatological units at some other centres like Madras and Lucknow if there be need to establish the same.

A procedure to be employed in carrying out clinical trials of liver extracts by the hæmatological units was submitted by Dr. J. C. Patel. It was approved and is attached as *Appendix II*.

Agenda item 7.—*To consider a request from Shri A. Tozhutaparamban of Cochin State regarding carrying out of clinical trials with sodium hypophosphites in the treatment of threadworms.*—

The Committee was of the opinion that there was not sufficient evidence of effectiveness of sodium hypophosphite in threadworms and hence the consideration for undertaking the trials of the drug was not recommended.

Agenda item 8.—*To consider a request from the Professor of Biochemistry, Indian Institute of Science, Bangalore, regarding carrying out of clinical trials with lemon-grass oil preparations.*—

The Committee recommended the Central Drugs Laboratory, Calcutta to undertake trials of the effectiveness of lemon-grass oil without any extra expenditure to the Indian Research Fund Association.

Agenda item 9.—To consider a request from the British Drug Houses (India), Ltd., Bombay, for the conduct of clinical trials with "Myanesin".—

The Committee decided to undertake clinical trials of Myanesin (B D H) and recommended that Dr. B. B. Yodh at the Grant Medical College, Bombay and the unit at the Seth G. S. Medical College, Bombay working on tetanus be requested to carry out trials on Myanesin in tetanus. The Committee also requested Lt.-Col. S. Narain, Assistant Director (Hygiene), Medical Directorate (D M S/5), Army Headquarters, New Delhi to ascertain whether he could arrange the clinical trials of this drug in operations in suitable military hospitals.

APPENDIX I.

Rules and Regulations for the Conduct of Clinical Trials of New Remedies under the Indian Research Fund Association.

1. *Objects and scope of work.*—The Therapeutic Trials Committee has been set up by the Indian Research Fund Association on the recommendation of the Clinical Research Advisory Committee to encourage and aid sound research on medicinal agents and to promote therapeutics by a thorough investigation of the usefulness and limitations of new drugs. The Committee will organize impartial clinical trials of biological and pharmaceutical agents which offer promise in the prevention, treatment or diagnosis of disease. The Committee with the voluntary and co-operative effort of pharmaceutical manufacturers, laboratory and clinical investigators and the medical profession will critically appraise the value of new drugs and disseminate information about them.

II. *Procedure to be followed.*—

(1) The Committee will invite and receive from outside sources suggestions regarding new products which are offered for the diagnosis, prevention and treatment of disease. Prescribed forms for the purpose of submitting requests to the Committee for clinical trials may be obtained from the Secretary, Indian Research Fund Association (*Form I*). If the Committee is of the opinion that an investigation is feasible and desirable, steps will be taken to request suitable investigators to undertake the work. This co-operative project will be sponsored by the Committee.

(2) The Committee will collect all information, concerning available investigators and about university departments, hospitals, research institutions and other suitable agencies for conducting clinical trials with special reference to their fields of particular interest and competence.

(3) The Committee will facilitate the co-ordination of efforts by investigators engaged in allied therapeutic problems. It will also seek advice from experts working in the same field. If necessary the Committee may arrange a conference on a therapeutic problem of interest and facilitate a prompt exchange of information on problems in which investigation is proceeding at different centres.

(4) After a preliminary examination of the data submitted by the manufacturer concerning a new product, if the Committee is of the opinion that it merits investigation, it will contact competent workers for their opinion according to paragraph 6 through the heads of the institutions. If the same product or similar products are manufactured in different names by more than one sponsor, the co-operation of all of them will be enlisted. The Committee will be under no obligation to initiate or participate in any investigation.

(5) It is very essential that information as to its nature, standardization, pharmacological action and toxic effects must be furnished to the Committee before a product can be considered for clinical trials.

(6) The Committee members will be asked to vote as to whether the request shall be accepted or rejected or deferred pending further discussion. In the case of rejection the firm submitting the request is informed and the reason for the rejection given. Rejecting a request for clinical trials does not mean that the problem is not worthy of investigation but may mean that there is insufficient information to reach a decision or it is not the type of problem best suited for supervision by the Committee or that it is not feasible to do a proper

investigation at that time. If the Committee votes to defer action, other outside experts may be consulted or additional information may be sought from the firm.

The following details must be insisted upon :—

(a) Full data concerning the composition of the agent.

(b) Physical and chemical properties, including method of assay, particularly those applicable to *in vivo* studies.

(c) Pharmacology (action, toxicity and fate).

(d) Results of any preliminary clinical studies.

(e) Conditions for which the drug is believed to be of value.

(f) Proposed dosage and method of administration.

(g) Any other relevant information.

(7) The investigators approached to undertake the trials, will be requested to submit their views on the following points in the form annexed (*Form 2*) :—

(a) A proposed plan of investigation.

(b) An estimate of the cost of the investigation.

(c) An estimate of the time required to complete the investigation.

(d) An estimate of supplies or special equipment which they wish to be supplied.

(e) If the proposed plan of investigation appears unsatisfactory to the Committee, the investigator will be given opportunity to modify the plan. If this is not acceptable to the investigator, the problem may be offered to another investigator. While the Committee will offer advice, if requested, it assures complete freedom to the investigator.

(8) The Committee may, after perusal of the views of the investigators, give suggestions regarding technique of the proposed trials. If investigation on the same or allied remedies is being conducted in two centres simultaneously, the Committee may lay down some uniform and standardized procedure for case records and treatment data to enable maximum benefit to be derived from such investigations and avoid duplication of work. The Committee will supply to each investigator selected for the purpose a *pro forma* which will have to be adhered to in case of each drug and a report submitted to the Therapeutic Trials Committee on all the points included in the *pro forma*.

(9) Reports shall not be sent for publication until reviewed by the Committee. The investigators have freedom to publish their results in any manner. No mention of the Committee may, however, be made in any publication unless the report has been specifically authorized and approved by the Committee.

If after consultation with the Committee, publication appears desirable, the investigator will be authorized to publish the results in his own name in a suitable professional periodical as a report to the Therapeutic Trials Committee of the Indian Research Fund Association. Before the report is published, the sponsor will be informed confidentially of the results.

(10) The mutual obligations shall be considered as completed with the termination of the individual contract and further investigation with the co-operation of the Committee must be subject to further contracts.

(11) The cost of research undertaken by the Committee shall be borne by the sponsor of the product. Funds supplied by the sponsor will be used entirely for research work barring incidental departmental charges necessary to carry on the activities of the Therapeutic Trials Committee. The Committee will act not only to secure investigations but also to evaluate the claims made for the products in the interest of therapeutic research and progress. As such, the Committee is not interested in any funds not actually expended in research and will return any extra money received from the sponsor of a particular investigation after completion. The expense of holding conferences and visiting various institutions and research centres will ordinarily be met by the Committee from funds sanctioned by the Indian Research Fund Association.

(12) On occasions, the Committee may on its own initiative undertake to secure the investigation of a pressing problem in therapeutics. In such cases the Committee will approach competent investigators and provide funds for this work either from grants by the Indian Research Fund Association or from manufacturers who may be interested in the problem.

INDIAN RESEARCH FUND ASSOCIATION.

THERAPEUTIC TRIALS COMMITTEE.

FORM 1.

Application for Clinical Investigation.

The undersigned hereby requests the Therapeutic Trials Committee of the Indian Research Fund Association to arrange for a clinical investigation of

.....

Attached to and submitted as part of this application is the available scientific information regarding this agent, including the following :—

1. *Composition.*—(If a single substance, give structural formula, if known; if a mixture, name each ingredient and indicate quantity of each in a given quantity of the drug. In the case of biologic products or similar agents, state source and method of preparation).

2. *Properties.*—(Include both physical and chemical properties, method of assay and, if known, a method for detection of the agent in body-fluids and tissues).

3. *Pharmacology.* (Include data on actions, toxicity and fate of agent).

4. *Other relevant laboratory data.*—(Include, where applicable, data on biochemistry, bacteriology, immunology and the like).

5. *Results of preliminary clinical studies.*—(If preliminary clinical trials of the agent have been made, the methods and results should be described and the names and affiliations of the investigators given).

6. *Conditions for which agent is believed to be of value.*

7. *Proposed dosage and method of administration.*

It is understood that this information will be transmitted to the Committee for consideration and, if a decision is reached to sponsor an investigation, the information will be transmitted to the prospective investigators.

.....
Date

.....
Applicant.

.....
Affiliation.

.....
Official title.

This space for Committee use only.

Approved :

.....
Secretary, Therapeutic Trials Committee.

.....
Date.

INDIAN RESEARCH FUND ASSOCIATION.

THERAPEUTIC TRIALS COMMITTEE

Form 2.

Date.....

This proposal is to be prepared by the investigator to enable the Committee to understand the investigation and its implications. The information may be submitted in other form if the investigator prefers, but it should cover the subjects suggested by the headings. It is understood that all statements are tentative and do not preclude adaptations as the project develops, provided the total sum is not exceeded without specific concurrence of the sponsors and notification of the Committee.

1. Name, official position and address of responsible investigator.
2. Subject of investigation :—
   (a) Name or description of product or products.
   (b) Field of investigation.
3. Questions to be answered by investigator.
4. Plan of investigation.
5. Requirements for investigation :—
   (a) Personnel (professional, technical and clerical assistants and approximate annual salaries).
   (b) Materials (equipment and supplies).
6. Funds desired from sponsors :—
   (a) Professional assistants.....Rs.
   (b) Technical assistants.....
   (c) Clerical and stenographic assistants.....
   (d) Expenses of patients.....
   (e) Equipment.....
   (f) Supplies.....
   (g) Project overhead.....
   (h) Contingent fund (not to include overhead) _____

   Total Rs. _____
7. Nature of any institutional collaboration in this work.
8. Concise description of facilities available for the investigation.
9. Suggest frequency and extent to which you would furnish progress reports to the Committee.
10. Indicate whether it will be necessary for you to contact the sponsor and why. (No contact will be permitted with the sponsor except for purposes indicated above).
11. Statement of expected time of beginning work and estimate of its duration.

.....
Responsible investigator.

Approved :

.....
Therapeutic Trials Committee.

.....
Official title.

.....
Sponsor.

.....
Official title.

.....
Date.

APPENDIX II.

For testing the potency of liver extract, the diagnosis of the type of anæmia must be established. The testing of the liver extract must be carried out in macrocytic anæmias only.

(1) Thorough history of the patient must be elicited and particular attention must be paid to the diet, economic status and infections present or past.

(2) Physical examination of all the systems (including the central nervous system must be done).

The case of anæmia must be investigated as follows :—

(1) **Full blood examination :—**

Red blood cells (must be below 1,500,000 per c.mm.)

White blood cells.

Hæmoglobin.

Reticulocytes.

Cell volume.

M.c.v. (must be above 110c μ).

M.c.h., m.c.h.c.

Van den Bergh reaction (quantitative).

(2) Sternal marrow should be examined and must show a megaloblastic reaction.

(3) If possible, G.A. should be done.

Investigations like icterus index, urine, stool, W.R. or Kahn should be done. Blood proteins, screening of chest should be done.

These base-line investigations should be completed within 4 to 5 days of the admission. The patient must be kept on a lacto-vegetarian diet which will not influence hæmopoises.

In each case the amount of liver extracts to be tested should be given in divided doses on the 1st day or in the first six days.

After the injection of liver extract has been given to the patient and during the base-line investigations, the reticulocyte count must be done daily. Subsequently, the blood count (r.b.c., w.b.c., Hb., cell vol., and reticulocyte) should be done on the 7th, 14th and 17th days. To judge the effectiveness of liver extract, the formula of Della Vida and Dyke (1940) may be used as criteria for an optimum response, along with other suitable criteria.

If the liver extract in a certain specified dosage gives the optimum response in at least four cases it should be considered effective.

Every new batch of liver extract prepared and submitted by each manufacturer should be tested.

The question whether any particular unitage should be assigned to liver extract should be further discussed.

One can adopt the criteria adopted by the Anti-anæmia Board of America or the unitage suggested by Emery and Hurran (*Brit. Med. Jour.*, 1945, p. 194). This question should be postponed till the working of the unit for one year.

MATERNITY AND CHILD WELFARE.

1. Enquiry into the pelvic type and its relationship to body-build in Indian women under Dr. S. Mitra at the R. G. Kar Medical College, Calcutta.

Turner's classification of the types of pelvis was taken as the basis for this work.

In antero-posterior skiagrams of the inlet of the pelvis taken by Thoms' method, the antero-posterior and maximum transverse diameters were measured, the error due to parallax being corrected by the use of a grid.

$$\text{Pelvic Index for Superior Strait} = \frac{\text{Antero-posterior diam.}}{\text{Transverse diam.}} \times 100$$

When pelvic index is less than 90 ... the pelvis is *Platypellic.*

When pelvic index is more than 90 but less than 94·9 ... ... the pelvis is *Mesatipellic.*

When pelvic index is more than 95 ... the pelvis is *Dolichopellic.*

Fifty-four subjects were examined. Forty of these were clinic subjects, and most of these were who conceived for the first time. The rest were recruited from middle-class families. The clinic subjects included a few from the low economic group. All the subjects except one hailed from Bengali parentage and a note was kept of their caste (if Hindu), dietetic habits and previous health.

Of the 54 subjects, there were 4 pre-pubertal (non-menstruating) girls who were expecting to have thier menarche very soon. It is desired to follow the changes in their pelvic architecture following on the attainment of womanhood.

A study of the skiagrams of the other 50 subjects showed the incidence of the following types and the frequency :—

Type.	Occurrence in : subjects.	Frequency percentage.
1. Platypellic ...	12	24
2. Mesatipellic ...	11	22
3. Dolichopellic ...	27	54

The American figures (Greulich and Thoms) are :—

Type.	Clinic women, per cent.	Subjects of high economic status, per cent.
Platypellic ...	35·2	13·5
Mesatipellic ...	27·2	13·5
Dolichopellic ...	37·3	73

2. Enquiry on anæmias of pregnancy under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Analysis of the data.

Up-to-date, a series of 96 unselected cases have been studied. Every case has been investigated thoroughly, both clinically and in the laboratory.

Eighty-seven of the patients were found to be suffering from anæmia and only 9 showed normal blood values. It may be remarked that in the hospital class of patients anæmia during pregnancy is unusually common and further that such patients may not complain of any symptoms.

I. An analysis of the data gathered from the 87 cases showed that 79 of them could be classified as suffering from the so-called pernicious anæmia of pregnancy. The results indicate that the only safe criteria for the diagnosis of this anæmia are (i) the presence of a true megaloblast in the peripheral blood and (ii) a megaloblastic marrow. The following table shows the relationship between these criteria and the (hæmatological) type of anæmia as revealed by the examination of the peripheral blood :—

Total 79 cases.

Type of anæmia			Megaloblasts in the peripheral blood.	Megaloblastic marrow.
Macrocytic :				
Orthochromic	15	...	16	In all, except one, where marrow was not examined but megaloblasts were seen in the peripheral blood.
Hypochromic	15			
Normocytic :				
Orthochromic	25	...	5	
Hypochromic	7			
Microcytic :				
Orthochromic	3	..	2	
Hypochromic	16			

Cell size as determined by m.c.v. Hæmoglobin saturation as determined by m.c.h.c.

It has been possible to show in this work that macrocytosis as judged by the colour index, mean corpuscular hæmoglobin, mean corpuscular volume or mean corpuscular diameter is not necessarily present in this anæmia as originally believed (Wills and Mehta, 1930). Normocytosis does not exclude the diagnosis of this anæmia, not even microcytosis ; further, the various other diagnostic features propounded from time to time, like extreme hypocholesterolæmia (Chatterjee, 1940; Nayer, 1942), increased red cell fragility (Elliot, 1944), spherocytosis, etc., are all unreliable. As it is always easier to spot out the megaloblast in the marrow than in the peripheral blood, the diagnosis of this anæmia in any pregnant female cannot be excluded without an examination of the marrow smear. It may be pointed out, that, in view of these new diagnostic criteria for this anæmia, opinions previously expressed on many of its aspects will have to be revised.

The other interesting features noted in the present series about this anæmia may be summarized as follows :—

(a) Hyperbilirubinæmia was present in 22 cases, but this was accompanied by a high reticulocyte count, urobilinuria and/or splenomegaly in only 2 cases. The hæmolytic variant of this type of anæmia is, therefore, encountered infrequently in Bombay and it may be deduced that precedent chronic malarial infection is not an ætiological factor in the majority of the cases in this part of India.

(b) Coincident ankylostoma infection or syphilis is not uncommon; the former was present in 17 patients and the latter in 10.

(c) A detailed investigation of the dietary habits (past and present) of these patients revealed that they had been consuming a defective diet for a long time; the obvious deficiency consisted of total and animal proteins. The finding was further supported by the demonstration of hypoproteinæmia in 57 out of the 79 cases.

(d) Histamina fast achlorhydria was discovered in only 2 of these cases, hypochlorhydria was present in the majority. In contrast to Addisionian pernicious anæmia, defective gastric secretion does not play a primary rôle in this anæmia.

II. The other 9 cases of anæmia belonged to the iron-deficiency group. The anæmia was microcytic type and the marrow was normoblastic.

3. Enquiry into the reproductive patterns of urban and rural populations under Dr. C. Chandra Sekar, Professor of Statistics and Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.

The collection of data has been completed in an urban area, situated in Central Calcutta, and a rural area in the Singur Health Centre, in Hooghly district. At present the information is being gathered of women living in South Calcutta. The latter area was selected for a second urban field as it has comparatively less conservative, more educated, and economically better-placed middle-class community, than the one in Central Calcutta. The enquiry covers all the women living in each of these areas between the ages of 12 and 50 years. The individual schedules filled of different communities uptil now are 2,072, 2,085 and 1,498 in Singur, Central Calcutta (Beniatola area) and South Calcutta (Ballygunge area) respectively. It is hoped to cover the present field by the end of October and to start a new one, if possible in a community of a different nature from November 1948.

Some main results of the analysis of the data collected are summarized below. It is seen that each of the population covered differs in many respects from the others, though the subjects are all Bengali women of the same group of age, i.e. 12-50 years.

I. Socio-economic factors.

(i) *Marital status of women.*—Though all the women of the age 12-50 years are included, Table I shows, that the percentage of unmarried women is least in Singur, and highest in South Calcutta, Central Calcutta remaining in between the two. Married women and widows are quite the reverse in proportion, while those separated are least in South Calcutta.

Table I.
Marital status.

Area	Unmarried.		Married: with husband.		Widow.		Separated.		Total Number.
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Singur	201	9·7	1,614	79·3	212	10.2	15	0·7	2,072
Central Calcutta	548	26·3	321	63·4	197	9·4	19	0·9	2,085
South Calcutta	485	32·3	921	61·5	87	5·8	5	0·4	1,498

The average age at marriage of the women in Singur is 11·6 years, Central Calcutta 13·9 years and South Calcutta 16·2 years. With urbanization and increased standard of education and social set up, the average age at marriage increases. Thus, an average girl gets married 5·6 years later in a community like the one in South Calcutta, as compared with her sister in the rural area.

Again, in Singur about 91 per cent of the women are married before the age of 19 years. The corresponding figures for Central Calcutta and South Calcutta being 43 and 22·6 per cent respectively.

TABLE II.

Percentage of marital status at different age-groups.

Area	12-14 years.			15-19 years.			20-24 years.		
	Married with husband.	Unmarried.	Widowed.	Married with husband.	Unmarried.	Widowed.	Married with husband.	Unmarried.	Widowed.
Singur	36·2	63·4	0·4	91·0	7·2	1·8	94·8	0·0	5·2
Central Calcutta	6·7	92·8	0·5	43·0	56·5	0·5	79·1	18·7	2·2
South Calcutta	0·1	99·9	0·0	22·6	77·4	0·0	71·0	27·7	1·3

	25-29 years.			30 years.		
Singur	93·7	0·0	6·3	76·1	0·0	23·9
Central Calcutta	88·8	3·6	7·6	75·7	0·9	23·4
South Calcutta	92·5	5·4	2·1	82·8	1·4	15·8

It is also interesting to note that the average age at marriage varies considerably in different castes of Hindu community as shown in Table III :—

TABLE III.

Average age in years at marriage among principal castes.

Area.	Brahmin.	Vaidya.	Kayastha.	Other castes.
Singur	12·9	Figure too small.		11·6
Central Calcutta ...	14·3	16·5	14·7	12·5
South Calcutta ...	15·7	18·1	16·5	14·2

Mean age at marriage has been increasing in recent years. In Table IV, individuals are classified according to the length of period that has elapsed since their marriage. Taking the record for the past twenty years it is seen that the average age at marriage has been steadily increasing in all groups.

TABLE IV.

Age at marriage according to the time in years that elasped since marriage.

Years:—	0–4	5–9	10–14	15–19	20 and above
Area.					
Singur	13·7	12·6	12·0	11·0	10·3
Central Calcutta ...	16·8	16·1	14·4	12·9	11·5
South Calcutta ...	19·3	18·1	16·2	15·4	13·3

(ii) *Literacy.*—In literacy South Calcutta tops the list. Illiteracy in husband is practically unknown in this community, while in women it is only 5 per cent. The figures for illiteracy for the Singur groups are 41·5 and 91·6 per cent respectively. College education is 1·2 per cent in husband in Singur and *nil* in women. In South Calcutta it is 62 and 10 per cent respectively.

TABLE V.

Educational status of the husband and wife in different areas in percentages.

Area.	Illiterate.		Primary.		Sec. & High.		College.	
	Husband.	Wife.	Husband.	Wife.	Husband.	Wife.	Husband.	Wife.
Singur ...	41·5	91·6	42·9	7·8	14·4	0·6	1·2	0·0
Central Calcutta ...	6·4	21·3	10·5	58·6	51·9	19·0	31·2	1·2
South Calcutta ...	0·1	5·1	0·9	45·8	36·1	38·7	62·9	10·4

(iii) *Occupation of husband.*—Occupation has a bearing on the educational status. Nearly three-fourth of the population are agriculturists in Singur, while in South Calcutta there are none of this occupation. The proportion of professional people are quite the reverse.

TABLE VI.

Occupation of husband in percentages.

Area	Agriculture.	Clerical.	Trade.	Skilled labour.	Public Administration.	Landlord	Teaching.	Law.	Medicine.
Singur	72·7	6·3	11·0	3·5	0·0	2·4	0·9	0·3	0·6
Central Calcutta	1·5	35·9	31·6	7·2	1·0	2·2	5·1	2·1	3·2
South Calcutta	0·0	40·4	18·7	3·8	8·0	3·7	8·5	3·9	4·5

(iv) *Economic status.*—Table VII gives the income per capita of the population in different areas. In South Calcutta nearly 50 per cent have an income over Rs. 40 per head, while in Central Calcutta less than one-fourth have this income and in Singur only 1 per cent are in this group. This will have an effect on the health, education and the social status of the community.

TABLE VII.

Income per capita in different communities.

Area	Rs. 20.	Rs. 21-40.	Rs. 41 and above.
Singur ...	71·9	27·1	1·0
Central Calcutta ...	17·8	59·9	22·3
South Calcutta	4·8	47·3	47·9

II. REPRODUCTIVE HISTORY.

A total number of 6,155 pregnancies was recorded in Singur, 5,102 in Central Calcutta and 3,384 in South Calcutta. Of these live-births were 5,945, still-births 113 and abortions 125 in Singur. In Central Calcutta live-births were 4,753, still-births 97 and abortions 252, while in South Calcutta live-births were 3,119, still-births 73 and abortions 192. This gives the abortion and still-birth rates per 1,000 pregnancies as 21·9 and 18·4 for Singur, 49·4 and 19·0 for Central Calcutta and 51·7 and 21·6 for South Calcutta respectively.

The average number of termination of pregnancies during the married life of a widow is 3·1. The average age at widowhood for Singur women is 24 years, for Central Calcutta 26 years and for South Calcutta 27 years.

TABLE VIII.

Total terminations as still-births and abortions and their rates.

Area.	Total number of termina-tions.	Total still-births.	Total abortions.	Rate per 1,000:	
				Still-births.	Abortions.
Singur ...	6,155	113	135	18·4	21·9
Central Calcutta	5,102	97	252	19·0	49·4
South Calcutta	3,384	73	192	21·6	56·7

For practical purposes if we consider a woman after 40 years of age to have completed her reproductive life, the number of pregnancies she has during this period is 7·5 for Singur, 6·3 for Central Calcutta and 6·0 for South Calcutta. No pregnancies are recorded under the age of 14, though 36 per cent of the girls below this age are married in Singur.

TABLE IX.

Average number of terminations of pregnancies at different ages of the mother.

Age of mother in years:—	12–14	15–19	20–24	25–29	30–34	35–39	40 and above
Area.							
Singur	0·0	0·6	2·2	4·0	5·7	6·5	7·5
Central Calcutta	0·0	0·6	1·8	3·5	4·5	5·6	6·3
South Calcutta	0·0	0·5	1·5	2·8	4·5	5·0	6·0

TABLE X.

Average number of live-births per married woman at different ages.

Age :—	12–14	15–19	20–24	25–29	30–34	35–39	40 and above.
Area.							
Singur ...	0·0	0·5	2·1	3·9	5·5	6·3	7·3
Central Calcutta	0·0	0·6	1·6	3·2	4·3	5·3	6·0
South Calcutta	0·0	0·4	1·4	2·6	4·2	4·5	5·6

Table XI shows that the average number of live-births within a period of 4 years of married life is 0·5 in Singur and 0·8 in South Calcutta, the reason for this being that the girls in rural areas are married earlier and hence for a part of their married life they are not mature enough to bear children. This is evident from Table X above where for the same age group 20-24 years, the number of live-births is 2·1 for Singur and 1·4 for Calcutta. In the latter community the girls are married much later.

TABLE XI.

Average number of live-births per married woman in relation to duration of married life.

Duration of married life :—	0–4	5–9	10–14	15–19	20–29	30 and over.
Area.						
Singur	0·5	1·5	2·9	4·3	6·3	7·5
Central Calcutta ...	0·6	2·2	3·1	4·5	5·4	6·9
South Calcutta ...	0·8	2·2	3·5	4·8	5·6	6·2

TABLE XII.

Percentage of nullipara, primipara and multipara in the two groups below the age of 25 years and above the age of 25 years.

Area.	Below 25 years of age.			Above 25 years of age.		
	Nulli-para.	Primi-para.	Multi-para.	Nulli-para.	Primi-para.	Multi-para.
Singur	34	28	38	3	3	94
Central Calcutta ...	32	29	39	6	8	86
South Calcutta ...	20	39	32	7	10	83

Family limitations.—Family limitation in the usually known sense is almost unknown in the rural area. With better education and with the added sense of responsibility brought about by education, the desire for restricting the family, however, seems to increase. This is evident from Table XIII which shows that only 0·3 per cent of the women attempted contraception in some form or other in Singur, while the figure for Central Calcutta is 13·2 per cent and for South Calcutta 34 per cent.

TABLE XIII.

Percentage of women that attempted or desired family limitation.

Area.	Attempted.	Desired but did not attempt.	Did not desire.
Singur ...	0·3	4·0	95·7
Central Calcutta ...	13·2	7·0	79·0
South Calcutta ...	34·0	2·0	64·0

Knowledge of contraception was satisfactory in more than 1/3 of the women in South Calcutta, while it is about 1/6 in the Central Calcutta and practically nil in Singur.

TABLE XIV.

Knowledge of contraception in percentage.

Area.	Satisfactory.	Unsatisfactory.	*Nil.*
Singur	0·4	0·3	99·3
Central Calcutta ...	15·8	3·7	80·5
South Calcutta ...	37·7	6·7	55·6

TABLE XV.

Attitude towards family size.

Area	Fatalistic.	Rational.
Singur	86·8	13·2
Central Calcutta ...	46·3	53·7
South Calcutta ...	31·4	68·6

Rational attitude towards family limitation gives an indication as to the wish of the women to use contraception as shown in Table XVI.

TABLE XVI.

W

Area.	Yes.	No.
Singur	12·4	87·6
Central Calcutta ...	44·8	55·2
South Calcutta ...	55·9	44·1

This report gives only the broad facts. Detailed information is being analysed and tabulated.

4. Enquiry on anæmias in pregnancy under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

The work of the enquiry was started in May 1948, The work at present is being conducted in two places: (1) Kaorapukur, a village 6 miles from Calcutta and (2) in Eden Hospital Antinatal Clinic, attached to the Medical College, Calcutta. Uptil now altogether 58 pregnant women have been examined, 32 at Kaorapukur and 26 at Eden Hospital. From a complete

hæmatological investigation these 58 cases can be classified into the following groups :—

		Cases.
1.	Normal blood picture ...	15
2.	Anæmic blood picture ...	43
	(a) Macrocytic anæmia ...	23
	(b) Hypochromic anæmia ...	4
	(c) Dimorphic anæmia ...	4
	(d) Normocytic anæmia ...	12

Of the 43 cases of anæmia, the red cell count was below 1·5 millions in two cases and between 1·5 and 2·5 millions in eight cases. The remaining 33 cases had anæmia of mild degree with red cell count between 2·5 and 3·3 millions. The incidence of these cases in the different months of pregnancy is shown below :—

Month.		Cases.
2nd	...	5
3rd	...	5
4th	...	7
5th	...	9
6th	...	11
7th	...	12
8th	...	5
9th	...	4

Thirty cases of anæmia have been put on specific hæmatinic treatment. Hypochromic anæmias are being treated with ferrous sulphate, macrocytic anæmias with folic acid or parenteral liver extract. Dimorphic anæmias are being given combined treatment with iron and liver extract or folic acid. Repeat examinations of blood have so far been made in 11 cases.

The work has been greatly hindered at the village treatment centre at Kaorapukur for the last two months due to monsoon. It is, however, expected that with the cessation of monsoon, the work will be carried out more vigorously and by the end of the financial year repeat examinations will be completed in all the cases under observation. It is needless to say that during this period it will also be possible to study a larger number of cases at both the centres.

So far progress made by the pilot enquiry appears to be encouraging.

Report of the Meeting of the Maternity and Child Welfare Advisory Committee on the 29th November, 1948 in the Senate House, Madras.

PRESENT.

Dr. J. Jhirad. (*Chairman*).
Dr. A. Benjamin.
Dr. G. Coelho.
Miss M. Craig.
Dr. C. R. Das Gupta.
Dr. N. A. Purandare.
Dr. D. M. Satur.
Dr. S. Pandit. (*Secretary*).

BY INVITATION.

R.B. Dr. A. C. Banerjea.
Lieut-Colonel Baiket Narain
Dr. E. Benjamin
Dr. B. C. Das Gupta.
Lieut-Colonel C. K. Lakshmanan.
Dr. A. R. Mehta.
Dr. H. M. Rajamanikkam.

Before beginning business of the Committee, members stood up in silence in memory of Mahatma Gandhi.

The Chairman pointed out to the Committee the importance of Maternity and Child Welfare Service in preventive medicine and suggested greater efforts be made by the members to stimulate interest in the advancement of research in this field. She requested the members to consider the agenda carefully with a view to keep the budget within the provisions.

I. CONSIDERATION OF PROGRESS OF WORK ON ENQUIRIES SANCTIONED DURING THE YEAR 1948-49.

(i) *Enquiry into the pelvic type and its relationship to body-build in Indian women under Dr. S. Mitra at the R. G. Kar Medical College, Calcutta.*

The Committee noted that 54 subjects had been examined and that the investigators had proposed to complete 250 cases by the end of the official year. In view of the interesting data already collected and their anthropological value, the continuance of the enquiry was recommended with a suggestion that investigators may restrict their work to Bengali women only and they should adopt the terminology suggested by Cauldwell and Moloy.

The provision in the budget under the scheme was modified as follows:—

		Rs.
(a)	Part-time clerk and typist @ Rs. 25 p.m. ...	300
(b)	One radiographer @ Rs. 60 p.m. ...	720
(c)	One peon at Rs. 30 p.m. ...	360
(d)	One nurse (lump sum allowance) ...	300
(e)	One doctor (lump sum allowance) ...	250
(f)	One anthropologist (lump sum allowance) ...	600
(g)	Contingencies	300
(h)	Cost of *x*-ray plates	4,500
	Total ...	7,330

(ii) *Enquiry on anæmias of pregnancy under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.*

The Committee noted that under this enquiry 96 cases had been investigated to date, and findings seemed to throw a new light on the subject of anæmias in pregnancy. The continuation of the enquiry was recommended.

(iii) *Enquiry into the reproductive patterns of urban and rural populations under C. Chandra Sekar, Professor of Statistics and Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.*

The Committee noted that this enquiry will be completed by the end of this financial year.

(iv) *Enquiry on anæmia in pregnancy under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.*

Dr. C. R. Das Gupta explained the work that had already been undertaken. In view of the findings of the pilot enquiry the Committee recommended that the detailed enquiry as outlined by him may be undertaken, and approved of the provision asked for, with the suggestion to include an allowance of Rs. 300 per annum for the Health Visitor.

II. Proposals for new enquiries.

The Committee considered the new proposals and placed them in the following order of priority :—

(i) *Enquiry into the incidence of rickets in children below five years of age in the city of Bombay under Drs. G. Coelho and R. F. Sethana and Mr. N. C. Datta at the Grant Medical College, Bombay.*

The Committee strongly recommended this enquiry and suggested certain modification in the budget estimate, namely the replacement of a part-time clerk on an honorarium of Rs. 25 p.m. and an addition of conveyance allowance of Rs. 600.

(ii) *Enquiry into the morbidity of children between the ages of* 1-5 *years (pre-school) in the Singur Health Centre area under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.*

The Committee felt that this type of work should form a part of health services in an area, but in view of the circumstances explained by Colonel Lakshmanan and the shortage of staff, the enquiry was recommended with the suggestion that the Medical Research Assistant should have experience in pediatrics and should be able to undertake regular home visits ; his/her allowance may be calculated at Rs. 250 per month. It was further suggested that instead of the two field assistants a health visitor may be appointed.

(iii) *Enquiry into the causes of maternal mortality in the city of Bombay under Drs. K. M. Masani and G. N. Mody at the Nowrosjee Wadia Maternity Hospital, Bombay.*

As the last statistical enquiry into the maternal mortality was undertaken 10 years back, the Committee felt the need of a fresh enquiry, in view of the recent advances in therapy and the influence of the increase of population and consequent overcrowding and food shortage, the enquiry is recommended with the omission of the pathologist's allowance and an addition of Rs. 400 for contingent expenses in connection with post-mortem and other investigations.

(iv) *Determination of the architecture of the pelvis in Indian women with special reference to its inlet, its shape, size and relationship with the external pelvic measurements and mechanism of labour under Dr. N. C. Sen at the Sanctoria Hospital, Dishergarh (Burdwan district).*

The Committee noted that this investigation into radiological pelvimetry also related to Bengali women. As the scope of this enquiry extended to a follow up of labour, it should prove a valuable corollary to the enquiry already in progress under Dr. S. Mitra in Calcutta. It was suggested that an anthropological study may be carried out at the same time, preferably by the same anthropologist who is associated with Dr. Mitra's work. The Committee recommended this enquiry and suggested an addition of an allowance of Rs. 600 for the anthropologist.

(v) *Enquiry into the vital losses of pregnancy and infancy in a rural population under Dr. Maltha Sen and Mr. K. Mathen at the All-India Institute of Hygiene & Public Health, Calcutta.*

This enquiry relating to rural population was recommended in view of of an earlier enquiry of a similar type carried out by Dr. L. Ghosh in an urban area.

III. At this stage Dr. Raja, Director-General, Health Services, attended the meeting and with the permission of the Chairman addressed the Committee giving a short summary of the evolution of the definition of maternal mortality.

In this connection he invited the attention of the Committee to the following circular recently issued by the Director, Health Services, West Bengal, to his subordinates revising the definition of maternal deaths: "The term maternal death shall include not only the death of mothers within one month after delivery as recognized at present but all deaths of mothers including expectant mothers from all causes of death associated with pregnancy and confinement occurring *before confinement*, *during confinement* and *within one month after confinement*. A brief list of causes of maternal deaths is also given, viz., (i) shock of confinement, (ii) haemorrhage (ante-natal and post-natal), (iii) toxæmias, (iv) puerperal sepsis, (v) anæmia, (vi) trauma and (vii) other associated causes, i.e. heart diseases, T. B., nephritis, etc."

Dr. Raja requested the Committee to give their considered view on the definition of maternal mortality. After discussion the Committee felt that in order to keep uniformity with other countries the term maternal mortality should include "deaths of women during pregnancy, labour and within 4 weeks of confinement and even later if the death is related to pregnancy, labour or puerperium. It was further suggested to classify maternal deaths as:—

(i) Conditions directly attributable to pregnancy, labour or puerperium.

(ii) Diseases which are aggravated by pregnancy, labour or puerperium.

For reckoning maternal mortality rate only No. (i), namely "conditions directly attributable to pregnancy, labour or puerperium" is to be taken into consideration.

The Committee recommended that the above suggestion be circularized for adoption by all Provincial Health Departments.

IV. The Committee considered the memorandum on the conduct of enquiries into infantile morbidity and mortality drawn up by the Sub-Committee which was appointed in December 1947.

The Committee approved of the memorandum and recommended its publication and circulation after the schedules had been passed by a statistician.

In view of the unreliable information on deaths, which is of no value from the research point of view, the Committee felt the urgency of proper certification of deaths by medical practitioners and recommended that the municipalities in the larger towns be requested to improve their system of certification of deaths.

V. Colonel Greval with the permission of the Chairman appealed to the members to help to collect sera from mothers of erythroblastotic babies, as there was an acute shortage of Rh-negative sera. He suggested that a request be sent to leading maternity institutions in the country to co-operate with their Provincial Research Laboratories by sending suitable sera which have already been grouped, as also cord blood smears.

VI. The Committee felt that it should be possible for municipalities of larger towns to have all maternal deaths investigated year by year and with this in view suggested the appointment of an Advisory Board of 2 or 3 senior obstetricians to collaborate with the health departments to formulate suggestions for improving maternity services.

VII. The Committee approved of the suggestions made by Dr. Coelho that greater publicity should be given to the work done by the Indian Research Fund Association and recommended that a popular bulletin be published for the public and summaries inserted in the various medical journals.

FILARIASIS.

1. Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Screening tests.

Altogether thirty-three compounds were tested for their action on *Microfilaria in vivo*. Of these, twenty-one compounds were received from the Medical Research Liaison Committee for India, London, five from Professor Bhide of Poona and seven from other sources.

Microfilaria were collected from three sources: (1) From blood collected at night from hospital patients and haemolysed with saponin immediately afterwards; (2) from blood collected in citrated saline solution at night and haemolysed the next morning; and (3) from freshly collected hydrocele fluid. The *Microfilaria* were suspended in a mixture of three parts of hydrocele fluid and one part of Ringer-Locke solution (containing 0·4 per cent glucose) and this suspension was so made that each drop contained at least 50 to 60 *Microfilariæ*.

The drugs were dissolved in Ringer-Locke solution and different dilutions were further made with the same solution. One drop of microfilarial suspension was added to 2 c.c. of the diluted solutions of the drugs in $4'' \times \frac{3}{8}''$ test-tubes and were kept at room temperature. The controls were kept in the standard mixture of Ringer-Locke solution and hydrocele fluid at room temperature. The hydrocele fluid was stored in ice-chest after collection. In some of the earlier experiments it was noticed that some fluids coagulated spontaneously in the tubes. This difficulty was overcome by keeping the fluid at room temperature after collection and if coagulation took place the uncoagulated portion was preserved in ice-chest and used subsequently. The temperature of the room varied from 75°F. (24°C.) in winter to 95°F. (35°C.) in summer. It was noticed that the *Microfilariæ* in the control tubes lived longer during the winter months of January to March than during the summer and the rains. *Microfilariæ* kept in the incubator at 37°C. died even earlier.

A few of the present set of experiments were set up in the day after the *Microfilariæ* were collected from the blood kept overnight but most of the experiments were put up by mid-night with *Microfilariæ* freshly collected from the night-blood.

Readings were taken every hour during the night for the first four or five hours and then every two hours till all the *Microfilaria* died in the solutions of the drugs as well as the control tubes.

It was noticed that the time required for the death of all *Microfilariæ* in the drugs as well as in the controls was as long as 70 hours in some cases. This determined the "minimum lethal concentration" of those drugs. But as the *Microfilariæ* in the control tubes did not live for 70 hours in any of the experiments, it was felt that this minimum lethal concentration and the time required would not give a true picture of the activity of the drugs for purposes of comparison. So the time in which almost all the *Microfilariæ* died in the experimental tubes while almost all the *Microfilariæ* were active in the control tubes was taken to compare the activities of the various drugs and their lethal concentration. The results are shown in the following tables :—

TABLE I.

Compounds received through the Medical Research Liaison Committee for India, London.

Name.	Lethal concentration.	Time in hours.
As 21	1—12,800	16
As 45	1—12,800	16½
As 55	1—1,280	10
Cyaninedye No. 863	1—128,000	21
M & B 647	1—12,800	43
„ 736	1—6,400	27
„ 776	1—12,800	42
„ 938	1—12,800	43
„ 983	1—12,800	41
„ 1364	1—6,400	27
„ 1453	1—12,800	27
Miracil D	1—12,800	9½
R. D. 301	1—25,600	12
„ 302	1—6,400	23
„ 304	1—12,800	10½
„ 315	1—6,400	23
„ 353	1—25,600	19
„ 408	1—12,800	49
„ 421	1—12,800	16
S.D.T. 779	1—12,800	16
Styryl quinoline 314...	1—12,800	13½

Experiments with other compounds are in progress.

Five compounds were received from Professor Bhide of Poona. These compounds are slightly soluble in water but are readily soluble in sodium-bicarbonate solution. The minimum quantity of sodium-bicarbonate required for solution was extra 10 mg. added to 10 c.c. of Ringer-Locke solution which already contained 15 mg. per cent.

TABLE II.

Name.	Lethal concentration.	Time in hours.
P-iso-amyloxy-arsonic acid	1—128,000	13½
O-iso-amyloxy-arsonic acid	1—128,000	13
O-n-butoxy-arsonic acid	1—128,000	13
P-iso-octyloxy-arsonic acid	1—128,000	13
P-n-butoxy-arsonic acid	1—128,000	13

COMPOUNDS RECEIVED FROM OTHER SOURCES.

Anthiomaline made by Messrs. May & Baker.

Cam-Aqui prepared by Messrs. Parke Davis & Co. It is a synthetic anti-malarial drug and its composition is 4-(3′-di-ethyl-amino-methyl-4′-hydroxy-anilino)-7-chloro-quinoline-di-hydro-chloride-dihydrate.

Chloroquin another anti-malarial drug made by the same firm. Its composition is 7-chloro-4 (4'-di-ethyl-amino-1-methyl-butyl-amino)-quinoline-phosphate.

Myostibin (antimony V hexonate) and tin gluconate prepared by East India Pharmaceutical Works, Ltd., Calcutta.

Paludrine M 3349 (old) and M 4889 (the new anti-malarial) both prepared by Imperial Chemical Industries.

TABLE III.

Compound	Lethal concentration.	Time in hours.
Anthiomaline	1—32,000	17½
Cam-Aqui	1—16,000	29
Chloroquin	1—128,000	9
Myostibin	1—12,800	16
Paludrine M 3349	1—409,600	19
Paludrine M 4889	1—13,107,200	24
Tin gluconate	1—6,400	11

Some of the compounds had marked action against *Microfilaria in vitro*, especially Paludrine, Miracil D, Cyanin dye No. 863, Styryl quinoline 314, and Professor Bhide's compounds.

2. Enquiry to test the usefulness of the intradermal and other tests for the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.

The antigen used in the tests during the period under report is a saline extract of adult *Conispiculum gandiense*. Naturally infected *Calotes versicolar* collected in and around the neighbourhood of the Institute were dissected and the adult worms found in them picked out and transferred to 0·3 per cent phenol saline. The worms were washed free of host tissue by repeated transfer to fresh quantities of sterile saline. They were drained, given a final washing in distilled water and dried in a desiccator over sulphuric acid in vacuum. The vacuum-dried residue was ground in a sterile mortar with enough normal saline to make a final dilution of 1 : 100, freezed and thawed alternately twice in the course of a day, heated for 4 hours at 56 C. and centrifuged at high speed for 30 minutes. The supernatant extract was sterilized fractionally at 56 °C. and filtered through candle. The final test for sterility was again put up and if sterile, phenolized (0·3 per cent), ampouled in lots of 1 c.c. and stored in the cold until use.

As controls, saline extract of round worms (*Ascaris lumbricoides*) prepared in an identical manner and serum of uninfected *Calotes versicolar* were used. These antigens were diluted to 1 : 100,000 just before testing or on the evening previous to the test, in which case the diluted antigen is ampouled and kept in cold storage until time of use. The dose injected was 0·01 c.c. intradermally on the volar aspect of the forearm of patients. A wheal resulted reaching its maximum in 15 minutes in positive cases.

During the period under report these tests were done in a total of 407 cases in the Government Clinic, Saidapet and the Government General Hospital, Madras. From a careful appraisal of the history, physical examination and the examination of blood for *Microfilaria*, the cases were grouped as follows :—

*(1)	Clinical filariasis		259
(2)	Doubtful cases (probably not filarial) ...		34
(3)	Control cases (not filarial)		114
	Total ...		407

**Microfilariæ* were demonstrated in 13 of these cases.

The mean dimensions of the wheal in mm. with the test and control antigens for these groups of cases is given in Table I :—

TABLE I.

	Clinically filarial cases.	Cases in which *microfilariæ* were demonstrated.	Doubtful cases.	Control cases.
Conispiculum extract ...	7	8	4	4
Negative *Calotes* serum	3	3	4	3
Ascaris extract ...	4	4	5	4

It is seen that the antigen prepared from *Conispiculum guindiensis* causes a much larger wheal in clinically filarial cases than either an *ascaris* extract or negative *Calotes* serum and that the wheal in clinically filarial cases is larger than in the control cases. There is no appreciable difference in the mean size of the wheal caused by either of the control antigens in filarial and non-filarial cases. In a certain number of cases the *Conispiculum* extract antigen was used also in a dilution of 1 : 10,000. In the clinically filarial cases the mean dimension of the wheal with the higher concentration was 11 mm. There was no increase in the size of the wheal in the control group.

Other observers using the *Dirofilaria immitis* antigen and canine serum for control have adopted a difference of at least 3 mm. in the size of the wheals caused by these two reagents as a criterion for recording the results as positive. The results of testing in these 407 cases on the same basis which is supported by the mean size of the wheals caused by the reagents used in the test are shown in Table II :—

TABLE II.

Percentage of positive reactors with Conispiculum *antigen* (*saline*).

	Negative *Calotes* : Serum control.	*Ascaris* antigen Control.
Clinically filarial	85·7	75·8
Cases in which *Microfilaria* were demonstrated	100	100
Doubtful cases	0	0
Control cases	2·8	3·3

In a proportion of cases undergoing treatment with Hetrazan a reaction which was positive at commencement changed to negative in 2 to 7 weeks.

The work is being continued.

3. Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona, and Dr. K. Venkataraman, Director of the Technology Department of the University of Bombay.

Antimonials being the drugs so far commonly employed in the treatment of filariasis, the first part of the programme has been concerned with the preparation of surface-active antimonials. *p*-aminolaurylanilide was prepared, but its conversion into the stibonic acid by the Bart or modified Schellar reaction was unsuccessful. *p*-aminobenz-dodecylamide (m.p. 120°C.-121°C.) was then prepared, and subjected to the Bart reaction. In this case the stibanilic acid, which is under examination, was obtained together with the stibonic oxide.

In view of the reported activity of 4-aminosalicylic acid in tuberculosis, the synthesis of 2-hydroxystibamilic acid has been undertaken. Starting from 3-nitro-*o*-anisidine, 4-nitro-2-methoxystibamilic acid has been prepared, and purified through its pyridinium salt, m.p. 187°C.-188°C. Further work on the acid is in progress. Also stibonic acids from 4-nitro-4′-aminodiphenylsulphone and 4-acetamido-4′-aminodiphenyl-sulphone have been obtained, and surface-active derivatives are under preparation. Continuing the work on antimony compounds, *p*-stibanilobenzoic acid and its pyridinium salt were prepared. From the pyridinium salt, the methyl ester (m.p. 214°C.), ethyl ester (m.p. 189°C.), *n*-propyl ester (m.p. 162°C.), and *n*-butyl ester have been obtained, the last two being new compounds.

Recently, certain cyanine dyes have been reported to possess marked anti-filarial properties. Salts of a typical cyanine dye with different types of wetting agents have been prepared in order to ascertain the influence of the wetting agents on the anti-filarial activity, if any, of the parent dye. The salts, except in the case of Igepon T, were obtained crystalline and analytically pure. The salts are sparingly soluble both in water and in olive and coco-nut oils. Among the different salts in this series, the salt from guaiacol sulphonic acid was most soluble in water (0·025 per cent) and those from sodium naphthalene β-sulphonate, Gardinol WA, BDT and Nekal BX followed in that order.

The cyanine dye, 1-ethyl-3 : 6-dimethyl-2-phenyl-4-pyramido-2-cyanine chloride (863), has been found to reduce *Microfilaria* in the cotton-rat, but has little or no chemotherapeutic effect against *W. bancrofti* (*Annals New York Acad. Sci.*, 1948, **50**, p. 119). The dye, for a sample of which we are greatly indebted to Dr. L. G. S. Brooker of the Eastman Kodak Co., is soluble in water, but not in olive oil. The dye forms salts with wetting agents; these have increased oil solubility, but there is a simultaneous decrease in water solubility.

The claims regarding anti-filarial potency made for "Hetrazan" pointed to the need for looking for anti-filarial action in drugs other than antimonials or organo-metallic compounds in general. Such compounds would be free from the effects of the heavy metals in body-assimilation. While 4-aminosalicylic acid has aroused great interest in the chemotherapy of tuberculosis, the compound lends itself to ready variation in structure and consequential changes in physical properties. From this point of view, available methods for its

preparation were examined, and a convenient procedure has been developed for its synthesis. Starting from 4-nitro-*o*-toluidine, a commercially available dyestuff intermediate, 4-aminosalicylic acid (m.p. 133° with decomp.) has been prepared in an overall yield of 60 per cent. New derivatives of the acid have been prepared. 3 : 3′-dihydroxyazobenzene-4 : 4-′dicarboxylic acid has been obtained from 4-initrosalicylic acid, an intermediate in the synthesis of 4-aminosalicylic acid. The soluble disodium salt of the azosalicylic acid has been prepared. The azosalicylic acid is of interest on account of its reducibility *in vivo* to two molecules of 4-aminosalicylic acid. A paper on " 3 : 3-dihydroxyazobenzene-4 : 4′-dicarboxylic acid and 4-aminosalicylic acid " will shortly be published.

REPORT OF THE MEETING OF THE FILARIASIS ADVISORY COMMITTEE.

Held at 9-30 a.m. on the 2nd December, 1948 in the Senate House, Madras.

MEMBERS PRESENT.

Dr. C. G. Pandit (*Chairman*).
Dr. B. B. Dikshit.
Dr. R. G. Dhayagude.
Lieut.-Colonel Jaswant Singh.
Dr. A. K. Mukerji.
Dr. C. K. Prasada Rao.
Dr. K. C. K. E. Raja.
Dr. Sangam Lal.
Dr. M. O. T. Iyengar (*Secretary*).

VISITORS PRESENT.

Dr. S. Banerjee.
Dr. P. S. Chinnaswami.
Bt.-Colonel Sir Ram Nath Chopra.
Dr. A. N. Duggal.
Dr. M. G. Kini.
Dr. Y. S. Narayana Rao.
Dr. I. M. Puri.
Major N. G. S. Raghavan.
Dr. K. S. Sanjivi.
Dr. N. K. Tampi.
Dr. T. S. Tirumurti.
Dr. K. V. Venkatraman.
Dr. N. Veeraraghavan.
Dr. B. B. Yodh.

1. The Committee considered the work carried out by the three enquiries in progress during 1948-49.

(*a*) *Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.*

Dr. Mukerji gave an account of the work done under this enquiry during the current year which consisted of screening tests with various compounds and the results obtained therefrom. He also formulated the plan of work for the next year with a view to continue the screening tests as well as the intradermal tests.

The Committee agreed unanimously to the continuance of the scheme and recommended to the Scientific Advisory Board the budget as put up. In regard to the request of Dr. Mukerji for promoting Dr. M. N. Sen, Research Assistant on the enquiry from the existing scale of Rs. 160-10-330 to the scale of Assistant Research Officer, viz. Rs. 275-25-500-E.B.-30-590, the Committee felt that the matter may be taken up when cases of other officers of the same grade are considered for promotion.

(b) *Enquiry to test the usefulness of the intradermal and other immunological tests for the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.*

Dr. C. G. Pandit spoke on the work done on this enquiry during the current year and Dr. Venkatraman outlined the programme of work proposed for the next year.

The Committee unanimously recommended the continuance of the enquiry.

(c) *Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. K. Venkatraman, Director of the Technology Department of the University of Bombay.*

Dr. Dikshit gave an account of the work done under this enquiry during the current year. It was mentioned that for the continuance of this work and to test out the toxicity of various drugs that have passed successfully through screening tests by Dr. Mukerji, it would be necessary to provide a large supply of mice and requested that provision be made in the next year's budget for this purpose.

Dr. Pandit suggested that Dr. Dikshit might raise a colony of mice in his laboratory and agreed to obtain an initial colony from different institutions in India. For looking after the mice, it was recommended that two attendants in the grade Rs. 30-½-35 be provided and Rs. 300 for contingencies.

With this modification the Committee unanimously agreed to recommend the request for the continuance of this important enquiry during the next year with the revised budget of Rs. 12,890.

2. *Work on Hetrazan by the Madras Provincial Research Society.*

Dr. Prasada Rao gave an account of the work done on the treatment of filariasis with Hetrazan at the Medical College, Madras in association with the staff of the King Institute, Guindy. He stated that Hetrazan gave good results in acute cases. Against *Microfilariæ*, it was observed that they disappeared in three to seven days after treatment. Some cases were tested with filarial antigens before and after treatment. It was noted that in the majority of cases tested, the antigen tests became negative. Doses of 1·5 mg. to 2·0 mg. were tried in several cases. In the case of *Microfilariæ* carriers, there was a disappearance of *Microfilariæ* in three days. In cases of lymphangitis although relapses occurred in many cases there was a general improvement and the intervals between attacks were much longer. He stated that his impression was that Hetrazan had lethal effect on *Microfilariæ* only and not on the adult worms as shown by the reappearance of *Microfilariæ* after some time. He observed good results in early cases of elephantiasis but failed to observe similar results in the more advanced cases. In Dr. Prasada Rao's view it was not necessary now to hospitalize the cases after instituting Hetrazan treatment.

The Committee expressed their appreciation of the work done by the Provincial Research Society and hoped that the work would be continued. They suggested that when trying the drug on chronic cases, the water displacement method of judging the reduction of the size of the elephantoid limb may be employed.

3. *Filariasis work done by the Malaria Institute of India, Delhi.*

Major Raghavan gave an account of the work done in Sriharikotta Island in Nellore district, Madras Presidency.

The Committee expressed their appreciation of the work carried out under the auspices of the Malaria Institute of India, Delhi, and expressed their desire that such investigations should be continued as facilities permit.

4. The Committee considered the proposal submitted by the Director, Malaria Institute of India, Delhi for the setting up of a unit to conduct field trials to ascertain the efficacy of Hetrazan and/or anti-mosquito measures for the control of filariasis. The general principles underlying the scheme were approved. The Director-General of Health Services explained that wherever the work is proposed to be conducted the Provincial Government concerned should be requested to make a contribution towards the conduct of such study. The Committee agreed in principle with the establishment of a unit for the proposed study and recommended to the Scientific Advisory Board to consider the implementation of the scheme in the manner suggested by the Director-General of Health Services.

INDUSTRIAL HEALTH.

1. Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta.

The four assistant research officers of the unit—the medical officer, the psychologist, the physicist and the chemist—had their training in their respective fields during the three months, October to December 1947, at the Institute. The work of the unit started in January 1948.

I. Effects of excess noise on workers.

It has been the unit's observation that in quite a number of industries like iron and steel, engineering and textile, the intensity of noise at the work-places often approach the 100 decibel mark and in certain cases, go beyond it. People working under such noisy environment are likely to be adversely affected.

(i) *Effect of noise on the auditory acuity.*—In order to elicit whether the low incidence of deafness (4·7 per cent) obtained in a preliminary experiment in the spinning and weaving departments of a jute mill is significant, a control group of workers in a less noisy department of the same jute mill is being examined for the auditory acuity.

(ii) *Effect of noise on efficiency.*—The object of the experiment is to determine the effects of the noise, found to be of the order of 97 decibels, on the efficiency of the workers.

The experiment is being carried out in a local jute mill (Adamjee Jute Mills) with eight weavers of normal auditory acuity as subjects. The results are to be computed on the basis of a comparison of their individual efficiencies under normal conditions of work (i.e. when they are working in the noisy environment) and under similar conditions but with the intensity of noise reduced. For the reduction of noise ear-defenders of a type are being used which, while eliminating sounds of high intensity, allow small sounds. Most of the variables in the experimental set-up are controlled. Arrangements have been made with the mill authority to keep the quality of raw materials, cloth, and loom-speeds constant. Unavoidable meteorological factors like temperature and humidity are checked regularly. To neutralize other unknown variables the unit of efficiency has been taken as low as practical, i.e. production in one hour. Similarly, to avoid any seasonal variations in the efficiency with and without the reduction of noise, the ear-defenders are used in alternate weeks.

A preliminary statistical analysis of six weeks' data was done. The Table below gives the amount of work done, with and without noise as well as the difference for each individual worker and for the group as a whole. The 2·18 per cent increase in efficiency with reduction of noise for the typical weaver is significant considering the consistent increase but falls short of statistical significance to the orthodox 95 per cent level. The preliminary experiment thus warrants a full-scale experiment which is being continued.

When the experiment is completed early next year and the results computed, it is expected that in addition to the basic objects of the experiment, additional pointers of importance might be brought to light.

As in similar experiments in Industrial Psychology financed by the Indian Research Fund Association in the past the research workers of this unit were faced with a number of problems. But for the team work amongst the unit and co-operation from the industry and the Institute of Hygiene, such a type of work in Industrial Psychology could not have been successful.

TABLE.

NOISE AND EFFICIENCY.

Experiment in a jute mill.

(Part I of the study).

Analysis of six weeks' data—4 weeks without ear-defenders, 2 weeks with ear-defenders (excluding Saturdays and broken hours.)

Weaver number.	Output—typical day—in inches.			Percentage efficiency—typical day.		
	With noise.	Without noise.	Difference: Without—With+	With noise.	Without noise.	Difference: Without — With +
213	5,099·41	5,198·97	+ 99·56	76·56	78·04	1·48
211	5,257·86	5,262·86	+ 5·00	78·94	79·02	0·08
210	5,069·60	5,326·29	+ 256·69	76·12	79·97	3·85
209	4,236·10	4,445·75	+ 209·65	63·60	66·75	3·15
208	4,265·67	4,304·90	+ 39·23	64·04	64·63	0·59
206	5,383·51	5,719·59	+ 336·08	80·83	85·87	5·04
220	5,043·11	5,088·01	+ 44·90	75·72	76·39	0·67
221	5,104·11	5,279·85	+ 175·74	76·63	76·26	2·63
Typical weaver	4,932·42	5,078·27	+ 145·85	74·05	76·24	2·18

II. INVESTIGATION INTO THE INCIDENCE OF LEAD INTOXICATION IN PRINTING PRESSES.

Lead is a long-recognized occupational hazard in printing presses, but practically no cases of lead poisoning have been reported from among the printing press workers in India. The Industrial Health Research Unit has, therefore, taken up this enquiry in one printing press to start with.

As the workers in the mono, lino and mono-casting departments are exposed more to lead fumes, examination is done for all the workers of these departments. A control series of examination will be done at a later stage on the workers of the binding department.

Each of the workers are examined for the following :—

(i) Physical examination with special reference to nervous, muscular and gastro-intestinal systems.

(ii) Basophilic aggregation test and stippling cell count of peripheral blood.

A modified Mccord's method of vital staining is used to stain the basophilic substance present in the red cells in ultra-microscopic quantities and Wright's stain for the stippling cells.

(iii) Estimation of concentration of lead in blood.

(iv) Estimation of concentration of lead in urine.

Modified titrimetric dithiozone method is being used. Lead dithiozone complex from ashed-blood or urine is extracted in chloroform, and the dithiozone used in this reaction is titrated against a standard lead nitrate solution.

(v) Analysis of air in working environment for concentration of lead to which workers are exposed.

Electrostatic precipitator (110 A.C. in put—15,000 volts output) and m.s.a. midget impinger are being used to sample the air. On an average 2·01 mg. of lead per 10 cubic meters has been found.

(Permissible limit is 1·5 mg. per 10 cubic meters).

A summary of results is given below :—

Concentration of lead in 100 g. of blood.			Concentration of lead in 1 litre of urine.			Basophilic aggregation (cells per 100 r. b. c.)		
Maximum obtained.	Minimum obtained.	Maximum permissible.	Maximum obtained.	Minimum obtained.	Maximum permissible.	Maximum obtained.	Minimum obtained.	Maximum permissible.
0·30	0·01	0·06	0·50	0·02	0·08	3	0·6	1·5

Summary of observations on lead hazard in one Government Press, Calcutta.

Period of Service.	Workers in the group.			
	Normal.	Sub-clinical.	Clinical.	Total.
Below 1 year	9	2	1	12
Between 1 and 5 years	5	3	...	8
Between 5 and 15 years	10	4	...	14
15 years and above	2	1	3	6
Total	26	10	4	40

This press is Government-owned and is one of the best managed presses here so far as the general sanitary conditions are concerned. It is proposed, therefore, to continue the investigation in another Government-owned press and also in some model presses after completing the work here.

Simultaneously, another enquiry into the incidence of lead poisoning in the soldering departments of factories is being conducted. About 24 workers have been examined so far out of which 2 clinical and 4 sub-clinical cases of poisoning have been detected. Further work is in progress.

III. Laboratory experiments to assess the toxicity of industrial dusts.

Industrial dust is one of the commonest occupational hazards. The toxicity of these dusts can be assessed in the field by clinical examination of the pneumoconiosis that develops in the workers or by controlled laboratory tests. As a preliminary to conducting elaborate field enquiries on large groups of workers in different industries, laboratory tests are commenced.

Suspensions of adequate quantities of 1 to 3 micron size dust particles (collected by "gravitating separation" method adopted from modified Stokes, calculation) are injected either intraperitonially or intravenously in guinea-pigs or rabbits respectively and the results observed in period from 3 months to 2 years. Five different types of dusts are injected and results awaited.

In the absence of a controlled dust chamber in the laboratory, a novel method of developing pneumoconiosis in laboratory animals by keeping them in the working environment is being tried.

IV. Relation of environmental warmth to comfort.

This investigation was begun on the 1st of October, 1947 and will continue till the 1st of October, 1948. Twelve different environmental factors involving physical comfort have been taken into consideration :—

1. Relative humidity.
2. Absolute humidity.
3. Heat content in b.t.u.
4. Dry-red kata rate of cooling.
5. Wet-red kata rate of cooling.
6. Evaporative cooling (kata).
7. Blue kata heat loss.
8. Air velocity as measured by blue kata.
9. Effective temperature.
10. Modified effective temperature.
11. Mean temperature of surroundings.
12. Mean comfort vote.

These will be correlated with comfort numbers on a 7-point scale recorded by twelve volunteers. Collection of data will be complete by 1st October, 1948 and the results and conclusions can be expected only after the statistical analysis and computation.

2. Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta.

Two jute mills and two printing presses were taken up and observations were made once a week at selected sampling points outside and inside various departments on temperature, humidity, light, air movement, etc. Dust-counts were taken in jute mills and found innumerable. The survey has been extended to a *dhal* mill, two workshops and two flour mills, besides the jute mills and the presses.

The observed data for a whole year for the jute mills and the presses are being analysed by the Section of Statistics. Diurnal variations in environmental

conditions in these industries have also been recorded on two days—once in winter and once in summer, at intervals of 3 hours.

Data on accidents, sickness and absenteeism, have also been collected from each department of the presses and the jute mills. These have to be correlated with environmental conditions. There is an indication of a marked inverse correlation between indoor lighting and accidents in the jute mills. Production figures are rather difficult to obtain. If they were available, they could also be correlated with environmental conditions.

Graphical charts are being prepared to show the variations in environmental conditions.

A general survey of housing conditions has also been carried out.

3. Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar.

This enquiry into sickness absenteeism due to accidents is the first of its kind in India. The enquiry was begun in March, 1948.

The broad outlines of work undertaken and on which the present investigation of accidents is proceeding are :—

I. *Study of all old accident records from* 1943 *onwards.*—Evaluation of all accidents, according to the causes of accidents, the agency causing accidents, disability and absenteeism, the accidents and the departments in the factory, the time factor, age of the worker and his caste and occupation.

Calculation of the injury-frequency and severity rates.

Calculation of absenteeism due to accidents.

Final assessment of disabling injuries and disability.

Direct cost of accidents compensation and medical expenses.

II. *Investigation of all individual accidents, as from* 1-8-1948.—In order to study all the factors, in the causation of accidents, six schedules have been prepared, and each accident analysed from data collected in the various schedules. These schedules are :—

Schedule I : Information regarding the individual accident, with full investigation notes on all points, including fatigue and accident proneness.

Schedule II : Information regarding the personal data, age, caste, training, education, etc.

Schedule III : Information regarding the injured worker and his present job.

Schedule IV : Information regarding the worker's opinion and his job.

Schedule V : Information regarding the injured worker and his home and social environments.

Schedule VI : Foreman's Accident Report on the worker.

I. Study of all records from 1948.

Only one comparative table of all relevant summarized data is presented for the year 1948.

The total number of accidents is 2,568, of which the reportable ones are 747 and the non-reportable ones 1,815.

The reportable 747 accidents are dealt with here, as they form the cause of absence for more than 7 days.

The total time lost due to all the accidents is 5,321 working days, while that due to the machinery alone is 2,510 working days, i.e. nearly 50 per cent of the accidents absenteeism is due to machinery.

The disabling injuries are classified for the whole group of skilled and non-skilled workers, as well as for the group of skilled workers as follows :—

Group.	Permanent.	Permanent partial.	Temporary.	Total.
Whole group	40	62	645	747
Skilled workers only	22	16	207	245

Nearly more than 50 per cent of the permanently disabled workers are the skilled workers. This is due to the carelessness and nonchalant mentality of the skilled worker.

The agency causing the disabling injuries are :—

		Total number.
(i)	Due to machinery	294
(ii)	Due to definite negligence of the worker	21
(iii)	Due to other workers	16
(iv)	Agencies other than machinery ...	416
	Total ...	747

For 1943, the injury-frequency rate is 13·23 and the severity rate 5·69.

A comparison of the accident statistics with the other available years being worked out, is not given here for the sake of brevity.

II. Investigation of all individual accidents as from 1-8-1948.

This relates to the present work on hand, from 1-8-1948 up to the end of September 1948.

Twelve accident cases have been followed in all their aspects, *vide* the six schedule mentioned above. Three hundred and thirty accident cases have been partially followed.

A comparative statement of the causes of accidents, in the month of August, for the years 1943 and 1948 is submitted below :—

Causes of accidents.	Percentage of total reportable accidents.	
	1943 (128)	1948 (113)
A. *Agency causing accidents :—*		
(i) Machinery	71·10	55·75
(ii) Hand tools	10·16	7·08
(iii) Vehicles	6·26	26·55
(iv) Chemical substances	5·48	5·31
(v) Hot substances	7·00	5·31
B. *Accident causes :—*		
(i) Unsafe working conditions in the factory	4·69	5·31
(ii) Unsafe act of the workers	83·82	88·50
(iii) Using unsafe or no equipment ...	4·69	2·67
(iv) Due to other workers	3·40	1·76
(v) Violation of safety rules in the factory	3·40	1·76

It will be noted that the unsafe act of the worker is responsible for the great number of accidents, thus showing the importance of personal factor in accidents.

C. *Classification of disabling injuries:—*

Month.	Permanent.	Permanent partial.	Temporary.
August 1943 ...	3	12	52
August 1948	...	39	45

4. Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow.

Introduction.

The investigation which commenced in July 1947 was undertaken with the object of studying the sources of fatigue and determining the most suitable conditions of work and efficiency.

The enquiry conducted last year had revealed certain peculiarities of the effects of work on the output of the workers. In order that these peculiarities might be satisfactorily explained a detailed investigation was undertaken with a view to making a more precise determination of the phenomenon of fatigue.

The enquiry continued.

Criterial performance.—In connection with the data of output, we started with the determination of *a criterion of performance.* We selected a short interval of half an hour (even though it would have been desirable to have a shorter interval, we had to fix half an hour because there is no mechanism in the mills by which shorter intervals could be recorded) during which the worker was supposed to record his 'ideal' performance and the efficiency of the worker was measured in terms of deviations from it. These *criterial levels* have been determined in the roving, spinning and intermediate sections. The following table gives the criterial levels in the various sections and shifts (in hanks) :—

Section.	Morning.	Day.	Night.
Roving ...	3·23	3·26	3·12
Intermediate ...	3·70	3·81	3·84
Slubbing ...	6·51	6·68	6·51

Relative efficiency ratio.—A second series of measures of output have been derived by calculating the ratio of the average to the maximum rate of the working of the machine. This may be termed the 'relative efficiency ratio'. These values were obtained from the production figures of two months and half (i.e. from 15th December, 1947 to 29th February, 1948). The following are the results :—

Per cent relative efficiency ratio.

Section.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
Roving ...	88·86	89·00	88·45	88·54	87·88	88·17
Slubbing ...	74·03	74·08	75·54	75·10	77·31	74·81
Intermediate	70·59	68·22	70·37	69·50	69·79	67·47

The figures show that the production has not reached the criterial level in the slubbing and the intermediate section, because in these sections the percentage of work does not go beyond 71 and 76 per cent respectively. In the roving section, however, the percentage is fairly high. It is possible that the efficiency level attained in the slubbing and the intermediate sections is lower than in the roving section perhaps because the following factors are operative :—

(1) The varying heights of the intermediate, slubbing and the roving machines require different bodily and postural adjustment by the worker. There is much greater strain involved in the slubbing section on account of the up and down movement of the spindles to which the worker has to keep on an adjusting.

(2) This postural adjustment, particularly the total bodily movement involved in bending and rising, is necessitated by frequent repair and mending of the thread-breakages in the slubbing section, as distinct from the roving section where the breakages can be repaired only by deft finger movements.

(3) The slubbing machines are located in the less lighted part of the shed.

(4) The space available for movement for the worker of the slubbing machines is comparatively smaller and appears cramped.

Variation of output in a shift.—The following are the average of the 'criterial production' taken at four different stages of the day (in decimals of hanks):—

Section.	2nd half hour of the shift.	Half hour before the interval.	Half hour after the interval.	Last half hour of the shift.
Roving :—				
Morning ...	3·23	3·10	3·16	3·07
Day	3·26	3·13	3·27	3·11
Night ...	3·12	3·11	3·05	3·03
Intermediate :—				
Morning ...	3·70	3·60	3·56	3·55
Day	3·91	3·69	3·77	3·53
Night ...	3·84	3·68	3·62	3·51
Slubbing :— ...				
Morning ...	6·51	6·39	6·48	6·36
Day	6·68	6·61	6·67	6·52
Night ...	6·51	6·39	6·29	5·96

The criterial production figures point out that :—

(1) The worker gives the maximum work in the earlier part of the day.

(2) The work in the first hour is poorer than in the second or third hour of the shift.

(3) There is a slight improvement in efficiency after the interval which is due to the rest pause.

Introspective report in variations.—The subjects report a renewed interest in work, disappearance of pain at the joints, and relaxation of the muscles. The refreshments probably make up for the loss of energy.

(4) There is a decline in efficiency at the end of the shift.

Day and night work.—The effects of day and night shifts have also been investigated. In this connection production figures of the two years, 1946 and 1947, were obtained. They were analysed and the average production of one machine per day was determined. The following are the figures of output in *hanks* in the various shifts :—

Slubbing.	Night.	Morning.	Day.
1946	8·60	8·65	8·64
1947	8·65	8·73	8·8

The figures would suggest unfavourable effects of night work. In both the sections and in both the years, there is poorer work in the night shifts. Ordinarily the morning shift gives the best work. In the morning shift itself, however, we noticed that there is lower efficiency and output in the first hour of the shift which is presumably due to the late warming up of the worker. This is further supported by the following average daily production of the months from January to July 1946 when only two shifts were worked :—

Shift.	January.	February.	March.	April.	May.	June.	July.
Morning ...	5·19	5·18	5·18	5·19	5·16	5·10	4·80
Night ...	5·14	5·14	5·16	5·15	5·08	5·07	5·81

Here again there is better work in the mornings in all the months.

Introspective report.—The report of the operatives throws further light on this point.

(1) During night shifts, they do fairly good work till about 12 o'clock. After that, they begin to feel sleepy. There is a feeling of uneasiness and lassitude.

(2) After struggle against sleep for a few hours, the inertia is broken at about 2 a.m. or 3 a.m. and there seems to be a temporary release of new energy.

(3) Early in the morning, there is increased lassitude, a kind of mild headache and irresistible feeling for rest.

These feelings may vary in intensity with different workers but they are present in most of them. The night work is done mostly sporadically with longer intervals of depression.

Experimental test-results and output.—Simultaneously, we introduced laboratory tests which would help us in understanding the nature of deterioration in output. The results of such tests may be used as supplementary data to confirm the findings of output studies. In order that the relation of output and test performance may be seen in a clearer perspective, the enquiry has been conducted in relation to two sets of data :—

(1) Output data arranged to show variations in rate of work for a complete working day.

(2) Results of steadiness and disc-dotting tests.

The following figures relate to the composite hourly output of the various sections and shifts (in hanks) :—

Hour :—	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.
Slubbing :—								
Day	13·14	12·75	12·46	11·75	13·04	11·96	11·71	12·57
Night	13·07	12·88	12·81	12·91	11·98	11·79	11·60	12·57
Intermediate :—								
Day	7·19	7·59	7·81	7·03	7·81	6·94	6·66	7·63
Night	7·15	7·35	7·48	6·71	7·42	6·79	6·63	6·96
Roving :—								
Day	7·29	6·87	7·05	6·20	7·08	6·29	6·16	6·33
Night	7·14	7·03	7·08	6·69	6·17	6·25	6·17	5·44

The important psycho-physical processes are muscular steadiness, quick reaction and visual motor co-ordination. With a view to testing these experiences we introduced three tests, viz. the ergographic test, McDougall's disc-dotting and steadiness tests. The following table gives the composite data of the score of the four individual workers of the sample.

Tests	Subject	Before work.	Interval.	After work.
Disc-dotting : (in hanks)	A ...	4·86	5·57	4·43
	B ...	3·14	3·57	4·86
	C ...	3·57	5·57	7·57
	D ...	6·29	5·14	5·57
Steadiness : (in hanks)	A ...	31·33	35·33	38·97
	B ...	33·67	34·67	38·83
	C ...	33·00	37·00	38·83
	D ...	27·67	34·67	38·67
Ergography (kilogramme-inches)	A ...	255·3	162·7	171·6
	B ...	190·8	149·7	129·0
	C ...	186·6	149·7	143·1
	D ...	229·5	150·0	132·9

McDougall's disc-dotting and steadiness tests were administered on the sample of workers at three different periods of the day in the factory situation, viz. before the beginning of the work, in the interval and after the work. The following figures give the average number of *errors* in the *two* tests at the three stages of the shift :—

Tests.	Before work.	Interval.	After work.
Disc-dotting ...	5·09	5·98	6.76
Steadiness ...	31·04	35·61	38.89

The following results emerge from the above :—

(1) There is a loss of concentration and accuracy in aiming in the fatigued state as is shown by the greater frequency of errors during the interval and after work.

(2) There is a weakening of the powers of inhibition as is indicated by the increase in the number of uncontrolled dots.

(3) There is greater lack of eye-hand co-ordination as is shown by the very great frequency of ill-adjusted and random aims (39 per cent errors).

CONCLUSIONS.

The more important results of our enquiry may be summed up as follows :—

(1) In spite of late ' warming up ' morning shift shows better performance on the whole.

(2) Compared to the morning and day shift, there is a steep rise in output in the first spell of the night shift. This is, however, soon counter-balanced by a comparatively poorer rate of work in the second spell (2.30 a.m. to 6.30 a.m.). Probably the earlier rise is brought about by the feeling that as much of work as is possible should be finished by 1.30 a.m. because afterwards the worker feels that the onset of sleep will impair his efficiency.

(3) Boredom is characteristic of the mental state of the worker in all the shifts in the last hour of the first spell and in the middle of the second.

(4) The point of maximum efficiency is reached in the second and the third hours of work in all the shifts.

(5) As a rule night work adversely affects the capacity of the worker and the quality of the work.

(6) The efficiency of the worker is found to be better in the winter months than in the summer (by 7·5 per cent).

(7) Organized rest-pauses and changes in posture are essential for checking fatigue from accumulating. More rest intervals in the night shift and better lighting may perhaps delay the onset of fatigue and counteract it.

(8) Since a high degree of muscular steadiness and co-ordination is required in the spinning processes, it is difficult for the worker to maintain them during the whole shift. In fact they are among the first to be perceptibly affected by fatigue.

(9) Distance from the mills seems to be significant cause of the early onset of fatigue.

(10) The work is affected by poor nutrition which naturally hastens fatigue.

5. Enquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose, Lecturer in Industrial Psychology, Calcutta University, Calcutta.

One hundred and forty tram driver-trainees have been tested. Of these 126 took all the tests, but the rest were not available for some of the laboratory tests.

The test scores have been statistically treated and on the basis of statistical findings the driver-trainees have been graded under five heads : Very suitable and should do well, suitable and may do well, indifferent and not likely to do well, doubtful but may be tried, and unsuitable and should not be tried. These classes are graded A, B, C, D, E, respectively.

The estimate of the trainer of these driver-trainees has been correlated with the test results.

The service records of those appointed by the Company are being followed up, taking particular note of the incidence of accidents.

To standardize the tests of accident-proneness arrangement has been made to test groups of drivers who have good service records for long periods and also those who have made a number of accidents in the same period of service.

Description of tests:—

1. A battery of intelligence tests composed of the following are used :
   (a) Dearborn form board test.
   (b) Passalong test.
   (c) Triangle form board test.
2. Colour series test.
3. Reaction-time test. Hipp chronoscope is used.
4. Steadiness test. The metal with hole of different diameter is used, and graphic records are obtained with the help of an electro-magnetic stylus and kymograph.
5. Fatigue coefficient. Finger ergograph is mostly used, but sometimes other types are also used.
6. Psycho-galvanic apparatus has been fitted and being tried on occasions. This is now in an experimental stage and hence the results are not being considered as yet in grading the individuals.
7. Interviews are arranged in certain cases. Personality questionnaire is used to note emotional maturity and stability.
8. Dotting test. This test is given to find out the extent of concentration of attention under conditions of distraction. An instrument specially devised by the Department of Psychology is used for the purpose.

To find the intelligence grading for each candidate the following statistical procedure has been followed :—

The *Z scores* for the test scores of each set of intelligence tests, viz. Dearborn, Passalong and Triangular Form, have been calculated and combined. The final combined scores for the individuals tested have been plotted. From the plotting the whole group has been divided into *five* sub-groups according to capacity. These five sub-groups have been graded as A, B, C, D and E.

To calculate fatigue index the amount of work done on ergograph has been calculated, and the scores plotted. For the purpose of grading five sub-groups have been marked off.

Similar procedures have been followed in case of steadiness, reaction-time and dotting (attention-concentration). As dotting test was given its final shape after a number of experimentation the curve for it has not been drawn yet as the cases observed under the final shape of the test are but few so far. Relative gradings for each batch have, however, been made.

Temperamental assessment is based upon a short interview. The grading is rather subjective so far, but attempt is being made to introduce a short standard questionnaire so that replies may be objectively considered.

Gradings of the first batch of 24 candidates are shown in the Table. The correlation between the final grading and the trainer's estimate as also that

TABLE

Psychological testing of tram driver-trainees

(Period of testing: March to May 1948)

Serial number.	Identity number.	Name.	Age (approximate).	Education.	Previous occupation.	Intelligence.	Steadiness.	Reaction time.	Fatiguability.	Attention concentration.	Temperament.	Final grading.	
					First batch of men tested.								
1	C/D 3	Ramayan Singh ...	23	Primary	Darwan	B	B	C	C	D	C	III	
2	C/D 4	Abdul Sahid	24	Can sign	Shop-keeper	C	C	C	B	C	C	III	
3	C/D 5	Md. Israil ...	23	Class II	Labourer	C	C	C	C	D	C	III	
4	C/D 6	Allauddin ...	23	Class V	Labourer	C	C	B	C	C	D	III	
5	C/D 7	Ulphat Miya ...	23	Class V	Packer	B	B	B	C	C	B	II	
6	C/D 8	Daspat Dewa ...	22	Primary	Carpenter	D	C	D	C	C	D	IV	
	C/D 9	(Absented himself from the tests).											
	C/D 10	Nandage Pande ...	23	Primary	Police Depart.	C	(Did not appear in other tests).						
7	C/D 11	Md. Behain ...	24	Class VIII	Shop-keeper	C	D	C	D	C	C	III	
8	C/D 12	Daspat Ojha ...	22	Primary	Darwan	D	D	D	C	C	C	IV	
9	C/D 13	Kalimuddin ...	25	Illiterate	Dock cooli	C	B	B	C	C	B	II	(Should
10	C/D 14	Md. Raffiq ...	21	,,	Shop-keeper	D	C	D	B	D	C	III	be given primary education before appointment).
	C/D 15	(Did not turn up).											
	C/D 16	(,, ,, ,,)											
11	C/D 19	P. Chowdhury ...	23	Class VI	Darwan	D	D	C	C	C	C	III	
12	C/D 17	J. Pande ...	25	,, ,,	,,	B	C	B	C	B	B	II	
13	C/D 18	A. Hussaianin ...	23	Primary	,,	D	D	D	C	C	D	IV	
14	C/D 21	Lalmohan Ojha ...	22	Class VI	,,	C	C	C	C	D	C	III	
15	C/D 22	Bhagbat Rai ...	26	Class V	Bus conductor	B	C	C	C	C	C	III	
16	C/D 23	T. Das ...	23	Can sign	Shop-keeper	C	B	B	C	B	B	II	
17	C/D 24	Karoo Singh ...	24	Illiterate	Key repairer	C	C	D	C	D	D	IV	
18	C/D 25	Ram Mahir ...	23	Class IV	Unemployed	C	D	D	C	C	C	III	
19	C/D 29	Gany Ram	22	Illiterate	,,	C	D	D	C	D	E	V	
20	C/D 41	M. A. Khan ...	22	,,	Peon	C	C	B	B	C	C	III	
21	C/D 42	Kamaleswar Singh	23	Primary	Farmer (Cultivator)	D	D	D	C	D	D	V	
22	C/D 44	Sasadhar Kar ...	24	,,	Cook	B	C	B	C	B	B	II	
23	C/D 45	Paresh Banerji ...	24	Class IX	Unemployed	C	D	C	C	C	D	III	
24	C/D 47	Sheik Elahi ...	24	Primary	(Peon) Bearer	D	C	B	C	B	B	II	
					Second batch of men tested.								
25	C/D 50	Ramkanta Jha ...	22	Class II	Ploughman	C	C	C	C	D	C	III	
26	C/D 51	Singheswar Prosad	23	Class V	Cultivator (?)	D	C	B	C	B	C	III	
27	C/D 52	Sudam Singh ...	27	Illiterate	Darwan	E	C	B	D	C	D	IV	
28	C/D 58	Prabhunath Singh	25	Class IV	Military	D	C	C	B	C	D	III	
29	C/D 60	R. Pandey ...	23	Class VI	Small business	C	C	C	C	D	D	III	
30	C/D 65	Lakshmi Jha ...	22	Class IV	Ploughman	C	B	C	B	C	C	III	

on.	Previous occupation.	Intelligence.	Steadiness.	Reaction time.	Fatiguability.	Attention concentration.	Temperament.	Final grading.
econd batch of men tested—concld.								
V	Unemployed	B	C	B	C	B	B	II
VI	Unemployed	C	D	B	B	C	C	III
II	Cooh (Labourer)	C	B	B	B	C	B	II
IV	Fitter	B	C	B	D	C	C	III
	Slop-keeper	C	D	C	C	D	C	III
VI	Machineman	B	B	B	C	C	B	II
ite	Bearer	C	C	B	C	C	C	III
V	Ploughman	C	C	D	C	D	D	IV
VI	Labourer	C	C	D	C	C	D	III
VII	Postman	B	D	D	C	C	C	III
V	Labourer	B	C	D	C	C	C	III
II	Darwan	D	D	D	C	D	D	IV
II	Ploughman	D	C	D	C	C	D	IV
	Unemployed	C	C	C	C	D	C	III
	Small business	D	D	D	C	D	E	IV
V	Ploughman	D	E	D	D	D	D	III
II	Darwan	C	C	D	B	C	C	III
V	Milkman	B	B	C	C	B	B	II
V	Milkman	C	C	D	C	D	C	III
	Unemployed	C	C	C	C	D	D	III
V	Milkman	D	D	D	C	D	D	IV
I	Darwan	C	D	D	C	C	C	III
I	Ploughman	D	C	D	C	D	D	IV
VI	Unemployed	C	C	D	C	C	C	III
V	Darwan	C	D	E	C	E	D	V
V	Darwan	C	D	D	C	D	D	IV

ld do well. II=Suitable and likely to do well. III=Average, but may develop
ay be tried. V=Unsuitable and *should* not be tried.

stimate of the trainer of the drivers in the training school=0·681.

he follow up results=0·624.

between the final grading and the follow up result (for six months) are noted in the Table. The correlations are not very high but are *significant*.

The plan of work so far has been to find out a suitable battery of tests for predicting good driving abilities. This will incidentally help to find out specific tests for accident-prone drivers. When norms for efficient, accident-free drivers are found the task will be easier for picking out the accident-prone drivers. More emphasis will now have to be laid on the preparation of suitable temperamental tests for measuring emotional stability and probing the unconscious as far as practicable within limitations. Arrangements are being made to test drivers who have been in service for more than two years and have been responsible for more than five accidents, with the Word-Association test given with the psycho-galvanic reflexes.

REPORT OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE.

Proceedings of the Fifth Meeting held in the University Buildings, Chepauk, Madras on the 29th November, 1948.

Members present.

Lieut.-Colonel C. K. Lakshmanan, Director, All-India Institute of Hygiene & Public Health, Calcutta. (*Chairman*).

Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay.

Professor K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta.

Lieut.-Colonel T. C. Pare, Deputy Director-General of Health Services, Government of India, New Delhi.

Dr. T. S. Avasthirmanian, City Health Officer, Madura.

R. B. Dr. M. G. Kini, 69, Marine Drive, Bombay.

Dr. M. N. Gupta, Deputy Chief Adviser on Factories (Medical), Ministry of Labour, Government of India, New Delhi (deputised for the Chief Adviser on Factories, Government of India).

Dr. M. N. Rao, Assistant Professor of Physiological and Industrial Hygiene, All-India Institute of Hygiene & Public Health, Calcutta. (*Secretary*).

Mr. S. C. Joshi, Dr. C. L. Kamal, Mr. N. S. Mankiker and Major V. M. Albuquerque were unable to attend.

By invitation.

Lieut.-Colonel A. N. Duggal, Director of Public Health, Bihar, Patna.

Major-General A. C. Chatterji, Director of Health Services, West Bengal, Calcutta.

Dr. E. Benjamin, Director of Public Health, Bombay.

R. B. Dr. A. C. Banerjea, Director of Medical and Health Services, United Provinces, Lucknow.

Lieut.-Colonel Barkat Narain, Director of Health Services, Delhi Province, Delhi.

Dr. A. R. Mehta, Deputy Director of Health Services, East Punjab, Simla.

Dr. D. V. S. Reddy, Professor of Physiology, Medical College, Madras.

Dr. M. V. Radhakrishna Rao, Assistant Director, Haffkine Institute, Bombay.

Lieut.-Colonel S. D. S. Grewal, Serologist to the Government of India, School of Tropical Medicine, Calcutta.

Dr. R. B. Lal, Professor of Epidemiology, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. D. K. Viswanathan, Assistant Director of Public Health (Malaria) Bombay Province, Poona.

Dr. R. K. Anderson, Field Director in India of the Rockefeller Foundation.

The Chairman welcomed the members of the Committee, the persons who were present by invitation and the visitors who attended the meeting. He explained to the members that the funds at the disposal of the Indian Research Fund Association were limited and requested the Committee to support only the more important enquiries.

Item 1. *–To consider the work carried out by enquiries sanctioned during the year* 1948-49 *and to make recommendations regarding the lines of work proposed for* 1949-50—

(a) *Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta.*—The work of the unit during the year was confined to four lines of investigation, viz.—

(i) Organic and psychological effects of excess noise on workers in jute textile industries.

(ii) The clinical and sub-clinical manifestations of lead hazard in printing presses and other industries.

(iii) Laboratory experiments to assess the toxicity of industrial dusts.

(iv) Relation of environmental warmth to comfort.

The proposed work for the next year included studies in rest pauses, occupational dermatitis and physico-chemical analysis of dust samples from industries.

The work of the unit was discussed at length and valuable suggestions were offered for future investigations, e.g. on the pathology of fibrous dusts, effects of city smoke, susceptibility to lead poisoning, etc.

The Committee recommended the continuation of the unit.

(b) *Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcuttta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta.* –Professor Subrahmanyan explained the progress of the work of his enquiry and indicated the broad outlines of the interesting conclusions that were being drawn. The figures collected so far were being analysed statistically. It was proposed to continue the work in other types of industries around Calcutta itself and not in the more distant areas or other provinces.

The Committee recognised the importance of these fact-finding surveys and recommended the continuance of the enquiry.

(c) *Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar.*—Though the enquiry was on the agenda of the Committee on two previous occasions, it was explained that due to various difficulties the medical officer who was to conduct the enquiry joined only in the beginning of the financial year and after defining the problem from the available statistics in the industry, the study of individual accidents commenced only in August 1948. It was expected that by the end of the financial year enough accident cases would be studied to give adequate information on the subjects of study, although the field covered may not be as extensive as originally planned.

The Committee agreed with the Chairman that an extension of the enquiry by another three months would give enough time and opportunity to do the statistical analysis.

(d) *Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow.*—The Chairman explained that the enquiry was completing the minimum period of two years it was planned for. He also explained the directive the Committee had given at its fourth meeting to Dr. Kali Prasad with regard to the programme of work. The Chairman discussed at length

the suggestions made by him and the correspondence on the details of work in this connection. The Committee went into the details of the report submitted by Dr. Kali Prasad and found that though some pains had been taken to collect figures from the factory records and the "Criterion of performance" studied under different conditions, very little attention had been paid to statistical considerations. It was feared under the circumstances that the usefulness of the enquiry would be very much less if the same type of incomplete work was carried on in textile industries in different parts of the country.

The investigation in three departments had been completed by October, 1948, and only that in the fourth, the weaving department, had to be completed. This could easily be done before the end of the year. The Committee agreed with the Chairman that intensive work done in one mill would achieve the desired object of study. The Committee therefore decided to terminate the enquiry as from March 1949, and consider the question of continuance of this type of enquiry in other textile mills, only after a full report of the studies so far made on the fatigue and efficiency in one textile mill was available.

(c) *Enquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose, Lecturer in Industrial Psychology, Calcutta University, Calcutta.*—The Committee noted the progress of the work done so far and recommended the continuation of the enquiry and the additional staff asked for.

Item 2.—To consider proposals for new enquiries.—

(a) *Enquiry into the personality factor in industrial fatigue under Professor H. P. Maiti at the Institute of Psychological Services, Patna University, Patna.*—There was an active discussion on the subject. It was generally agreed that the object of the enquiry was noteworthy and that the results of the enquiry would give valuable information of use to the Industrial Physician in his pre-employment and pre-placement examinations and to the personnel departments in industry. But there was some difference of opinion whether the industrial medical services in our country were advanced well enough to make use of the results of the proposed research, which might be premature. But it was argued that the incapacity to use the results of the enquiry should not be confused with the necessity of acquiring the knowledge which was of such importance to Indian industries. The Committee after discussion agreed to recommend the enquiry.

(b) *Enquiry into the prevalence of Byssinosis in Cotton Textile Industry in South India under the direction of Dr. T. S. Adisubramaniam, Madras Public Health Service, Madras.*—The Chairman placed before the Committee an application for a grant-in-aid which he had received from Dr. Adisubramaniam on the day the meeting was held. He explained to the Committee that Dr. Adisubramaniam was not aware of the procedure for applying to the Indian Research Fund Association for grants for enquiries and hence the delay in submission of the proposal.

Byssinosis, an occupational disease due to cotton dust, was a recognised condition in countries especially where the working environment was not very satisfactory. The sanitation in cotton textile industries in India was anything but desirable and it was felt that the incidence of this disabling occupational disease —Byssinosis—must be fairly high. In spite of the fact that the proposal had been placed before the Committee late, the members felt that the enquiry was worth taking up and strongly recommended the same.

Item 3.— Other business.—

The Chairman reminded the members that on previous occasions the Committee had recommended the purchase of a mass chest radiographic equipment. The Scientific Advisory Board had recommended the same to the Government of India, but necessary funds for the purchase of the equipment had not yet been provided. The Committee resolved that in view of the urgent necessity for acquiring the equipment in connection with industrial health research work, the Government be approached to expedite the matter.

RABIES.

REPORT OF THE MEETING OF THE RABIES ADVISORY COMMITTEE.

Held in the Senate House, Madras, on the 30th November, 1948.

The Committee met at 9-30 a.m. on the 30th November, 1948.

PRESENT.

Lieut.-Colonel M. L. Ahuja. (*Chairman*).
Lieut.-Colonel S. D. S. Greval.
Dr. S. R. Pandit.
Dr. K. V. Venkatraman.
Dr. N. Veeraraghavan.
Dr. A. G. Brooks. (*Secretary*).

BY INVITATION.

Dr. Gurkripal Singh.
Dr. S. S. Pillai.

1. The Rabies Advisory Committee at their meeting held in December 1947 had recommended that claims with regard to 'culture' vaccine as prepared at the Pasteur Institute, Coonoor, should be independently investigated by other workers. A report on the work in this connection carried out at the Central Research Institute, Kasauli formed the basis of discussion. The Committee were of the opinion that the results obtained with 'culture' vaccine were decidedly encouraging and resolved that the experimental findings presented at the meeting should be included as an *Appendix* to this report. It was also resolved to place on record the Committee's appreciation of the work conducted at the Central Research Institute, Kasauli.

2. It was decided that a strain of street virus should be fixed for the purpose of using it as an infecting agent in antirabic experiments. Dr. Veeraraghavan undertook to do this. This strain would not be used for the preparation of antirabic vaccine in any laboratory in India. It would be kept as a standard infecting strain for tests of evaluation of antirabic vaccines.

3. The Committee noted that the Government of West Bengal had decided that 5 per cent antirabic vaccine should be used as standard antirabic treatment in West Bengal. The Committee recommended that all antirabic centres in India should adopt uniform strength and dosage of vaccine.

4. The question of maintenance of elaborate statistics of people undergoing antirabic treatment was considered in detail. It was resolved that antirabic statistics, in their present form, should be maintained by all Pasteur Institutes in India, but that separate figures for Europeans and Asiatics were no longer necessary. It was also decided that every endeavour should be made to collect data regarding groups of persons, some of whom had received antirabic treatment and some had not. Both groups should be observed for at least six months for purposes of comparison.

5. A note on trials of antirabic serum conducted at the Central Research Institute, Kasauli was considered. Results of preliminary experiments with antirabic serum received from Lederles Laboratories, U.S.A., and of unconcentrated antriabic horse serum prepared at Kasauli were discussed. Indications are that serum prophylaxis is of some value in preventing rabies.

6. The Committee noted with pleasure that the Government of India had agreed to defray the cost of recurring expenditure of a Rabies Research Centre to be opened at Kasauli. The Director of the Central Research Institute, Kasauli was requested to ask the Pasteur Association of India for the grant of Rs. 1,50,000 which was promised in 1946, on condition that Government accepted this liability. The Chairman pointed out that there had been a rise in the cost of building material during the last few years and that it may be necessary to ask the Pasteur Association of India for an enhanced grant.

APPENDIX.

Research work during the year 1948.

The Rabies Advisory Committee of the Indian Research Fund Association at its meeting held on 9th December, 1947 recommended that Dr. Veeraraghavan's findings with regard to 'culture' virus vaccine should be investigated independently by all interested workers who had facilities to do so. During the course of the year the following experiments have been carried out in this connection at the Central Research Institute, Kasauli.

A. Antirabic vaccines.

Experiment No. C/1/47.—To ascertain whether Coonoor 'culture' vaccine is of *any* value.

Eighteen guinea-pigs were given 3 doses of 0·5 c.c. each of the culture vaccine, subcutaneously, at intervals of 3 days. Twenty-one days after commencement of treatment, they were given a challenge dose of fixed virus, subdurally, in a dose of 0·2 c.c., in varying dilutions as noted in Table I.

Table I.

Dilutions of fixed virus.

	10^{-4}	10^{-5}	10^{-6}
Treated ...	6/6	6/6	2/6
Controls ...	3/3	3/3	3/3

Note.—Numerator—Deaths from rabies
Denominator—Total animals
} These symbols are used throughout this report.

In all these experiments the same number of animals was originally used in each of the treated groups. Differences in the final denominators recorded are due to deaths from other causes which have been excluded from the figures.

Comment.—'Culture' vaccine appears to be of some value. Unfortunately an end-point was not reached in controls.

Experiment No. C/2/47.—To compare the antigenic value of 'culture' vaccine with that of Semple 5-per cent vaccine and a Semple vaccine diluted to the same opacity as the 'culture' vaccine. (It was found that 'culture' vaccine was equivalent in opacity to 0·5 per cent Semple vaccine).

This experiment was carried out in mice, 20 g. to 25 g. in weight. They were given 6 doses of 0·25 c.c. of vaccine intraperitoneally, on alternate days, followed by two more doses 10 and 12 days after the 6th dose. Ten days later, the mice were given a challenge dose of fixed virus 0·03 c.c. subdurally, in varying dilutions. The result is shown in Table II :—

Table II.

Vaccine.	Dilution of virus.			
	1 : 1,000.	1 : 5,000.	1 : 10,000.	1 : 20,000.
Semple 5 per cent	0/6	0/6	0/6	0/6
Semple 0·5 per cent ...	0/6	0/6	0/6	0/6
Culture ...	0/6	0/6	0/6	0/6
Controls (not treated) ...	4/6	2/6	1/6	1/6

Comment.—All the three vaccines tested are capable of protecting mice against an L50 subdural infecting dose.

Experiment No. C/8/48.—Same as the previous experiment but using mice of 10 g. to 12 g. in weight. Forty mice were divided into two groups, one of which received Semple's 50 per cent vaccine and the other 'culture' vaccine. Both vaccines were diluted 1 in 10 before injection. Of this 0·25 c.c. was injected intraperitoneally every other day; total six doses. Challenge dose was given two days after completion of treatment. It consisted of intracerebral injection of 0·03 c.c. of fixed virus mouse brain—the diluent used being 10 per cent normal horse serum.

TABLE III.

Vaccine.	Dilutions of virus :					
	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}
Semple 5 per cent ...	6/6	4/5	6/6	...	...	...
Culture ...	2/5	1/6	1/3	...	...	...
Control ...	...	3/4	3/3	4/4	2/2	2/4

Comment.—The indications are that "culture" vaccine affords better protection than Semple 5 per cent vaccine but such experiments will have to be repeated several times before a definite opinion can be expressed.

Experiment No. C/5/48.—Two groups of mice 20 g. to 25 g. in weight were treated with Semple 5 per cent and culture vaccine respectively. The dose vaccine administered was 0·25 c.c. intraperitoneally, every other day; total 6 doses. Two days after completion of treatment a challenge dose of street virus in varying dilutions was given intramuscularly to all animals. Result is shown in Table IV:—

TABLE IV.

Vaccine.	Dilutions of virus :				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Semple 5 per cent ...	2/4	0/4	0/6	0/5	0/5
Culture ...	2/6	0/5	0/5	0/5	0/6
Controls ...	1/4	2/4	0/4	1/4	0/4

Comment.—Street virus is too variable to give reliable results in mice. The same street virus as was used in this experiment had previously killed 2 out of 2 guinea-pigs in a dilution of 1 : 100, yet control mice given 1 : 10 dilution escaped.

Experiment No. C/1/48.—To compare the antigenic value of 'culture' vaccine Semple 5 per cent vaccine and Semple vaccine diluted to the same opacity as 'culture' vaccine (=0·5 per cent Semple vaccine). Monkeys were used as test animals. They were given a daily dose of 2 c.c. of the vaccine subcutaneously for 7 days. Two weeks after completion of treatment, the animals were given a challenge dose of 0·4 c.c. of 1 : 10,000 fixed virus intracisternally. Result is shown in Table V :—

TABLE V.

Semple 5 per cent ...	11/12
Semple 0·5 per cent ...	12/12
Culture ...	6/10*
Controls ...	6/6

*Two animals died of other cause.

Comment.—'Culture' vaccine would appear to afford somewhat better protection than the other two vaccines tested.

Experiment No. C/2/48.—Same as the previous experiment but Semple 0·5 per cent was not included and the challenge dose given was a 1 : 50,000 dilution of fixed virus. Result is given in Table VI :—

TABLE VI.

Semple 5 per cent	12/12
Culture ...	12/12
Controls (1 : 50,000)	6/6
Controls (1 : 100,000)	2/3

Comment.—Neither of the vaccines protected monkeys in this experiment against a challenge of fixed virus, given intracisternally.

Experiment No. C/3/48.—Same as previous experiment but a locally prepared 'culture' vaccine was included and two test doses used as shown in Table VII. Monkeys were used as test animals :—

TABLE VII.

Vaccine.	Infecting dose of fixed virus.	
	1 : 50,000	1 : 100,000
Semple 5 per cent	6/6	5/5
Culture (Coonoor)	4/6	5/6
Culture (Kasauli)	5/5	6/6
Controls ...	4/5	4/5

Comment.—The experiment is a complete failure as control animals fared better than treated. The contradictory results of the above experiments using monkeys as test animals, well illustrate the need for extreme care in drawing conclusions. The first experiment (C/1/48) which appeared to give differential results is nullified by the two subsequent experiments in which a much smaller challenge dose was given, the strain of virus, the mode of infection, technique and operations being identical in all these experiments.

Experiment No. C/4/48.—Repetition of experiment as in monkeys but guinea-pigs were used as test animals. Treatment consisted of 1 c.c. intraperitoneally every other day for 6 doses. Challenge dose consisted of 0·2 c.c. of fixed virus the titre of which had been previously ascertained in guinea-pigs. Unfortunately, this challenge dose failed to kill any of the animals and a second stronger challenge dose was, therefore, given to all animals 16 days later. Note that all animals *including controls* were given a second challenge dose. Result is shown in Table VIII.

TABLE VIII.

Semple 5 per cent	...	0/10
Culture (Coonoor)	...	0/10
Culture (Kasauli)	...	0/10
Controls ...	...	8/8

Comment.—A high degree of immunity is conferred by all the three vaccines in guinea-pigs treated as above.

Experiment No. C/6/48.—An indirect approach to the problem was made by immunizing two groups of sheep, twenty in each group, with Semple's 5 per cent vaccine and Coonoor 'culture' vaccine. Twelve days after completion of treatment, the sera of animals of each group were pooled separately and tested for their *in vitro* neutralization titre, by mixing equal quantities of various dilutions of serum, with 1/1,000 dilution of fixed virus. The mixture after incubation for ½ hour was injected subdurally in guinea-pigs. The results are given in Table IX:—

TABLE IX.

Vaccine.	Final dilution of serum.			
	1/4	1/8	1/16	1/32
Semple 5 per cent ...	2/2	2/2	2/2	2/2
Culture ...	1/2	0/2	0/2	2/2
Control (normal sheep serum) ...	2/2	...	...	...

Comment.—The results suggest that culture vaccine provokes a better antibody response than Semple's vaccine. This type of experiment is to be repeated several times before a definite opinion can be expressed.

B. ANTIRABIC SERUM.

Claims that antirabic serum is of considerable prophylactic value have recently been revived in America.

Through the courtesy of Dr. C. G. Pandit, Secretary, Indian Research Fund Association, we received antirabic serum raised in rabbits from the Lederles Laboratories, U.S.A. on 23-8-48. Independently antirabic serum in a horse had been prepared at this Institute the previous year and this was available for comparative tests.

The following preliminary experiments with American serum were carried out:—

Experiment S/1, dated 31-8-48.—A sheep was given 10 c.c. of U.S.A. serum intravenously and 2 hours later infected with fixed virus intracisternally (0·5 c.c. of 1 : 100 dilution as is used in routine passaging). A second dose of the serum was administered 6 hours later and after that twice daily. The sheep developed symptoms of rabies in 6 days (normal period) and died on the 8th day.

Comment.—There was no prolongation in incubation period or in period to death.

Experiment S 2, dated 31-8-48.—This experiment was exactly on similar lines to the above, except that a rabbit was used. The rabbit was given the ordinary dose as in routine passaging (0·2 c.c. of 1 : 100 dilution intracisternally) and serum intravenously 5 c.c. twice a day, the first dose of serum being given 2 hours before infection. As in the case of the sheep, there was no prolongation in incubation period or in period to death.

Experiment S 3/48, dated 25-9-48.—Guinea-pigs were infected subcutaneously with 0·5 c.c. of a 5 per cent suspension of street virus. Half an hour later the area was infiltrated with 2·00 c.c. of serum. Result is shown below :—

		Days of death.
U.S.A. serum	2/8	28, 30
Kasauli serum ...	5/8	30, 49, 50, 52 and 54.
Controls (not treated) ...	8/8	Average 17 days.

Note.—Final result will not be available till 25-12-48. (3 months after infection.)

Experiment S/4/48, dated 21-10-48.—Guinea-pigs were infected subcutaneously with street virus (first passage sheep) and infiltrated at varying intervals with Kasauli serum. U.S.A. serum was not used in this experiment as guinea-pigs were not available in sufficient numbers. The serum was administered ½ hour, 6 hours, 24 and 48 hours later to the different groups of guinea-pigs. Eight animals were used in each group and 4 untreated controls were also infected.

	½ hour.	6 hours.	24 hours.	48 hours.
Serum, result on 24-11-48 ...	7/8	6/8	6/8	0/8
Controls	4/4	...	...	...

Final result will not be available till 21-1-49.

Experiment S/5, dated 13-9-48. *In vitro comparison between U.S.A. (rabbit) serum and Kasauli (horse) serum.*—Equal parts of fixed virus (1 in 100 dilution) and the sera were mixed together, incubated for an hour and injected subdurally into guinea-pigs. The result is given below:—

	Final dilutions of serum :			
	1 : 8	1 : 16	1 : 32	1 : 64
U.S.A. serum	1/2	1/2	2/2	2/2
Kasauli serum	Not done	1/2	0/2	2/2

Comment.—Experiments of this type are to be repeated till sufficient numbers are obtained.

C. Miscellaneous experiments on rabies.

M/1/47, dated 28-7-47 : *'Nerve blocking' effect of cobra venom in rabies infection.*—On the analogy of the reports of some Russian scientists that a fatal dose of hydrocyanic acid could be given to an anæsthetized 'nerve blocked' rabbit, without ill effects, it was decided to test the 'blocking' effect, if any, of cobra venom in rabies infected animals, the venom being given previous to subdural infection. The venom was found to have no 'blocking' effect, the control and envenomized animals died in the same period.

M/2/47, *dated* 3-2-47: *Filtrability of rabies fixed virus.*—It has often been claimed that the virus of rabies is filterable. Fixed virus, ground as fine as possible with silver sand, was not found filterable through an L_3 candle, rabbits given intracisternal injection of the filtrate escaped infection.

M/3/47, *dated* 5-11-47: *Infectivity of C.S.F. of sheep dying of fixed virus infection.* –Two sheep given routine intracisternal infection of fixed virus were used in this experiment. The C.S.F. of each sheep was passaged by subdural inoculation into two guinea-pigs daily. One out of the 14 guinea-pigs injected, acquired infection 5 days after the sheep had been infected. Passage on all other days from the 2nd to the 7th day even when both sheep were moribund, were negative. The C.S.F. of a sheep dying of fixed virus infection is, therefore, very feebly infective.

Experiment M/4/47, *dated* 11-12-47: *Infectivity of fixed virus stored for 2 years at* 4°C. to 7°C.—Two fixed virus rabbit brains preserved in 50 per cent neutral glycin and stored in the cold room at 4°C. to 7°C. for one and two years respectively were passaged into rabbits. The first proved infective and there was no prolongation in incubation period or in period to death. The 2-year-old brain was non-infective.

Experiment M/5/48, *dated* 5-5-48: *Keeping properties of fixed virus at* 0°C.—The brain of a sheep dying of fixed virus infection was ground up into a 20 per cent emulsion in 50 per cent glycerine and stored at 0°C. This brain emulsion has been titrated at intervals in guinea-pigs with the following results :—

Date	Dilutions of virus :		
	1 : 250,000	1 : $2\frac{1}{2}$ m.	1 : 25 m.
5–5–48 ...	2/2	1/2	1/2
8–7–48 ...	2/2	0/2	0/2
10–8–48 ...	2/2	2/2	0/2
10–9–48 ...	2/2	2/2	0/2
8–10–48 ...	2/2	1/2	...

This experiment is still in progress.

Experiment M/7/48.—Attempts to grow street virus in the presence of living organisms—Baker's yeast, *P. notatum*, *P. camemberi* and *A. oryzæ*—have proved unsuccessful.

PHARMACOLOGY.

1. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratory, Jammu.

Essential oil-bearing plants.

Carum carui *Linn.*—*Caraway* (*Vern.-Zira*).—Caraway consists of the dried ripe fruits of *Carum carvi*, an erect biennial herb indigenous to and cultivated in North and Central Europe, chiefly in Holland and in Central Asia. In India, it is cultivated as a summer crop in the hills of Baltistan, Kashmir, Kumaon, Garkwal, etc., at an altitude of 9,000 ft. to 12,000 ft. above sea-level. In Kashmir the plant commonly occurs in Astore, Drawa, Gurez, Skardu and it is also cultivated in the valley. The plant is cut when the fruit is ripe, which is then separated by thrashing. It is largely used in both the Indian and Western medicine as stomachic and carminative. Caraway oil is also used as carminative. It is also used for flavouring wines and scenting soaps in perfumery.

For the distillation of the essential oil caraway seeds growing in Skardu, Gurez and Baghanpura were procured and the properties of oil obtained were studied. These are compared with the standards laid down in British Pharmaceutical Codex, 1934.

Table.

Locality :—	Baghanpura.	Gurez.	Skardu.	B.P.C. standard.
Altitude above sea-level	5,500 ft.	7,900 ft.	8,700 ft.	...
Yield of the oil ...	4·3 per cent	8·8 per cent	8·3 per cent	3·5 to 6 per cent.
Specific gravity ...	0·9095	0·8902	0·8907	0·910 to 0·920
Refractive index ...	1·491	1·486	1·485	1·485 to 1·492

A perusal of the Table shows that the caraway growing in various parts of Kashmir gives good yield of the oil which is up to B.P. and B.P.C. standard.

Origanum vulgare *Linn.* (*Wild Narjram ; Vern.-Satra*).—The plant grows wild commonly in the temperate Himalayas at altitudes from 6,000 ft. to 12,000 ft. above sea-level. In Kashmir it is commonly found from 5,000 ft. to 9,000 ft. in the forests in the valley.

It is reported to be eaten at certain places as a pot herb. In the indigenous system of medicine the plant is used as aromatic, stimulating, and in the treatment of diarrhœa, rheumatism and ear-ache.

The plant was collected from the neighbourhood of Gulmarg and on steam-distillation the essential oil had the following characteristics :—

Yield of the oil	...	...	...	0·76 per cent.
Specific gravity	...	...	...	0·933 at 15°C.
Refractive index	...	...	...	1·477 at 20°C.

Cherophyllum villosum *Wall* (*Vern.-Jangli Gajar*).—It is a perennial erect herb often found in the temperate Himalayas at altitudes from 5,000 ft. to 12,000 ft. In Kashmir the plant grows abundantly in pine forests and the roots are eaten by the local people as wild carrot. The whole flowering plant was steam-distilled and the essential oil obtained had the following characteristics :—

Yield of the oil	...	...	...	0·98 per cent.
Specific gravity	...	...	...	0·97 at 15°C.
Refractive index	...	...	...	1·463 at 20°C.

Achillea millefolium *Linn.* (*Vern.-Momadru, Chopandiga*).—It is a common plant found in the temperate Himalayas at altitudes of 6,000 ft. to 11,000 ft. In Kashmir it is found growing in abundance as a wild weed near the cultivated fields or forest banks around the valley. The flowering heads of the plant were collected for the distillation of essential oil which was obtained only in traces.

Eryngium caeruleum *Bich.* (*Vern.-Dudhali*).—It is a medium-sized erect herb growing in Kashmir valley at altitudes of 5,000 ft. to 6,000 ft. The root of the plant is taken as an aphrodisiac and nervine tonic. The plant was collected from neighbourhood of Srinagar but on steam-distillation it also yielded traces of essential oil.

Anthemis noblis *Linn.* (*Vern.-Babuneke-phul*).—It is a small herb growing wild and is also cultivated in the temperate Himalayas for the medicinal properties. In the Tibbi system of medicine it is collected locally but on steam-distillation only traces were found. The physical properties of the oils from the above three plants could not be studied as sufficient quantity of the oil could not be obtained.

Dubosiosia species.—*Dubosiosia myoporoides* and *D. leighardii* grow wild in New South Wales, Australia and are reported to contain two to three per cent of the total alkaloids which consist mainly of hyoscyamine and hyoscine. During World War II the Australian Commonwealth developed and improved this natural source for the production of these alkaloids. The Bureau of Plant Industry in Australia are extending the cultivation and improving the species by hybridization so as to obtain a higher content of the active principles.

Dubosiosia myoporoides and *D. leighardi* are not indigenous to India. Samples of the leaves of the two species of *Dubosiosia* were procured from the Australian Commonwealth and on analysis were found to contain 1·8 per cent and 3·7 per cent of total alkaloids respectively, more than five times as compared with those of *Atropa belladonna* and *A. acuminata* alkaloids.

Seeds of *Dubosiosia* sp. have also been procured through the courtesy of Trade Commissioner of Australian Commonwealth in India and these are being cultivated at different suitable localities to see if the plant can adopt itself to the Indian soil and climatic conditions. If the trial cultivation is successful the leaves of this plant can form a rich and constant source of these alkaloids.

Male ferns of Kashmir.

Male ferns is one of the oldest anthelmentic drugs known and was used by the ancient physicians, Pliny and Calen. It is administered in the form of extract filix-mas for eradication of tape-worm infection in man and livestocks.

The British Pharmacopœial drug is derived from the rhizomes and frond bases of *Dryopteris filix-mas* (Linn.) Schott., a fern indigenous to Great Britain.

D. marginalis A. Grey which is found in Eastern and Central United States and North to Prince Edward Island forms the source of American male fern.

D. filix-mas and *D. marginalis* are not indigenous to India but the other ferns belonging to the *Dryopteris* (Lastrea) *filex-mas* complex grow wild in the Himalayas in general and in the mountainous ranges of Kashmir in particular. These ferns are :—

Dryopteris rastnarai, etc. (Diels) C. chr. *D. blandfordii* (Hope). C. chr., *D. odontoloma* (Moore). C. chr., *D. ramosa* (Hope). C. chr. and *D. marginata* (Wall) Christ.

Samples of these ferns were collected in order to find out whether the above-mentioned ferns growing wild here can be substituted in place of the official fern and can be used in the manufacture of expensive male-fern extract imported from abroad in considerable quantity.

D. odontoloma occurs widely in the fir zones as an undergrowth throughout the Kashmir valley. It is more common on the southern aspect of the forests retaining good moisture at altitude of 5,500 ft. to 6,500 ft. above sea-level.

Five samples of *D. odontoloma* and one of *D. marginata* were collected from the various localities in Kashmir.

All the tests prescribed for the examination of the official rhizomes by the B.P. 1932 and U.S.P. XII were done and the figures compare favourably. Thus the percentages of total ash and acid insoluble ash are well below the prescribed limits and calcium-oxalate crystals are not present in any sample. The percentage of filicin in the rhizomes varies from 2·1 to 3·03 and the percentage of filicin in the extractive varies from 25·6 to 28·4 except in one sample of *D. odontoloma* obtained from Pahalgam. The limits of percentage for filicin in rhizomes and in extractive mentioned by the B.P. and U.S.P. are not below 1·5 per cent and not below 24 per cent respectively. Thus, the male-fern growing in Kashmir are up to B.P. or U.S.P. standard.

Indian wild cherry bark.

Wild cherry bark is the bark of *Prunus serotina* Ehrhart (family *Rosaceæ*), collected in autumn, when it is most active, is carefully dried and stored. The tree grows wild in North America, and most of the bark of commerce comes from Virginia, Indiana, North Carolina and Michigan.

The drug is popularly employed to relieve cough in phthisis and bronchitis of various types. The mild sedative action of the drug is generally attributed to the small quantity of hydrocyanic acid present.

Prunus serotina is not indigenous to India, but there are several species of *Prunus* which are either cultivated for their fruits or grow wild in India. An attempt was made to investigate if any of these species could form a good substitute for the official bark used for medicinal preparations.

The bark of *Prunus amygdalus* Baily (*Vern. Badam*) *P. avium* Linn. (*Vern. Gilas*), *P. armeniaca* Linn. (*Vern. Khubani*). *P. cerasus* Linn. (*Vern. Jangali gilas*) were analysed.

The last two species grow wild at 5,000 ft. to 7,000 ft. above sea-level, while the other species are cultivated extensively in the Kashmir valley for their fruits. Various parts of the plants of the above-mentioned species are generally used in the indigenous system of medicine.

The bark was collected from the stem and branches in the autumn season, dried and analysed.

As a result of these analyses it has been found that whereas the percentage of total ash and acid insoluble ash is generally above the prescribed limits, the percentage of alcohol extractive is lower than the official bark in most of the samples examined.

Rhamnus virgatus *Roxb.*—The work on *R. virgatus* was started last year in order to see if the plant could form a substitute for *R. purshiana* the source of *Cascara sagrada* an extremely useful anthracene purgative. The chemical investigation as well as trials on rabbits and puppies have already been reported.

During the period under review, trials of *R. virgatus* on animals were continued. The drug was administered to guinea-pigs in the form of dry extract. This animal was selected because it has been found to be particularly suitable for assay of anthraquinones. No cathartic effect was produced in guinea-pigs even with $\frac{1}{2}$ g. of the extract.

Finally, the drug was administered to human volunteers in the form of dry extract and bark. The dosage employed was two to three times more than the official. No cathartic effect was reported by these volunteers.

As a result of chemical investigations and experiments on different animals as well as human volunteers it has been concluded that :—

1. The bark of *R. virgatus* is chemically inferior to the official bark.
2. It does not possess any cathartic action.
3. It cannot, therefore, form a substitute of the official plant.

2. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.

It is gratifying to note that the Indigenous Drugs Enquiry, a most important branch of study in India, has again been sanctioned after a temporary break of two years. Owing, however, to the unsettled condition of the country the procurement of genuine crude drugs of suitable maturity, which is the starting material for our investigations was much hampered, and considerable effort had to be made in organizing the preliminaries. Through the kind co-operation of the Director, Forest Research Institute, Dehra Dun, the Conservator of Forests, Government of Assam, etc., it has been possible to procure the requisite amount of some of the crude drugs.

Dregea volubilis, a drug proposed to be taken up during the present year is still to be procured.

The work during the year includes the following :—

(a) *Dæmia extensa* Linn.—(i) The nature of action of an alcoholic extract of *Dæmia extensa* on the uterus has been under investigation. It seems likely that the contraction ring starts at the upper pole of the uterus and gradually spreads to the lower. Conclusive experimental evidence still remains to be had. (ii) The value of *Dæmia extensa* and the influence of vitamin C on its action in experimental paralytic ileus is under investigation. (iii) Progesterone is known to antagonize the action of pituitrin on the uterus. Since progesterone and vitamin C content in *Corpus leuteum* run parallel, experiments have been undertaken to observe the influence of progesterone on the uterine action of *Dæmia extensa* has been on clinical trials in maternity hospitals as regards to its ecbolic action. Reports are awaited.

(b) *Rauwolfia serpentina*.—It is well known that the extract of *R. serpentina* is used extensively in the treatment of high blood-pressure. With a view to find a suitable method for the estimation of alkaloids of *Rauwolfia serpentina* in body fluid after oral administration several methods were tried. The urinary elimination was first taken up for investigation. The recovery of the total alkaloids after adding a known quantity of the same to the urine was tried by precipitating the alkaloids and subsequent extraction with chloroform. Attempts to measure the alkaloid content of the chloroform extract after evaporation were, however, not successful as the percentage of recovery was very small. Eventually it was noticed that the total alkaloids give a beautiful bluish green fluorescence when exposed to ultra violet irradiation. This property of the alkaloids was utilized in quantitatively measuring the amount of alkaloid excreted in the urine after oral administration. From the results so far obtained it appears that after the first dose the elimination of the drug in urine is almost negligible. After administration, however, of several doses of the drug, the quantity eliminated in the urine increases and this increased elimination may be detected within one and a half hour of the test dose. Further studies are in progress. All the fluorometric measurements in these experiments were made with the help of Lumitrone.

(c) *Cissampelos pareira* Linn. contains about 1 per cent of the total alkaloids. Further chemical investigations as to the identification of the different alkaloids present and their subsequent separation and isolation with complete study of their chemical properties are in progress. Preliminary pharmacological studies indicate that the alkaloids tend to lower the blood-pressure and increase the frequency of respiration. Atropine appears to influence the fall of pressure and the respiratory frequency, thereby tending to attribute a cholinergic character to the drug.

(d) *Zizyphus jujuba* Lam.—A hydrochloride of an alkaloid extracted from the stones of the fruits was obtained through the courtesy of Dr. B. Roy of Astanga Ayurved Vidyalaya, Calcutta.

The hydrochloride did not melt even at 261°C. but gradually darkened probably due to decomposition. The hydrochloride is almost insoluble in absolute alcohol, ether and chloroform.

Pharmacologically the alkaloid appears to slightly raise the carotid blood pressure, while markedly depressing the intratracheal pressure. With a boiled solution of the alkaloid the carotid pressure does not appear to be influenced to an appreciable extent and the intratracheal pressure is only slightly depressed.

(e) A drug identified as *Lippia nodiflora* Rich was obtained through the courtesy of Dr. B. Mukerji, Director, Central Durgs Laboratory. It was reported to contain an alkaloid. The drug was extracted by one per cent hydrochloric acid, rectified spirit and also by the usual lime extraction method. No alkaloid could be detected in any of these extracts.

REPORT OF THE FIRST MEETING OF THE PHARMACOLOGY ADVISORY COMMITTEE.

Held in the Senate House, Madras, on the 29th November, 1948.

The Committee met at 9-30 a.m. on the 29th November, 1948.

PRESENT.

Bt.-Colonel Sir Ram Nath Chopra (*Chairman*).
Dr. B. B. Dikshit.
Dr. B. N. Ghosh.
Dr. J. C. Gupta.
Dr. B. Mukerji.
Dr. B. B. Yodh.
Mr. P. M. Nabar (*Secretary*).

IN ATTENDANCE.

Dr. J. C. David.
Dr. B. P. B. Naidu.
Dr. J. C. Patel.
Dr. J. C. Ray.
Dr. D. N. Roy.

Before the agenda was taken up for consideration, a question was raised as to the scope of the work of the Pharmacology Advisory Committee. The following resolution was passed.

"The Pharmacology Advisory Committee after considerable discussion regarding the scope of the Committee recommends to the Scientific Advisory Board that it should define as to what enquiries would come under its purview".

The items on the agenda were then taken up *seriatim* and discussed.

Item 1.—*To consider work done by the existing enquiries and to make recommendations regarding their continuation or otherwise during* 1949-50.

(i) *Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratory, Jammu.*

The Committee recommended that the enquiry should be continued during the next year.

(ii) *Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.*

The Committee recommended that the scheme should be continued during the next year. While making the above recommendation, the Committee observed that in future research workers on indigenous drugs should concentrate intensively on detailed pharmacological action of the active principles of indigenous drugs which have already been recognized and on which preliminary work has been done.

Item 2.—Any other business.

Two other enquiries, which were not included in item no. 1 of the agenda, were also considered :—

(i) *Enquiry to investigate bactericidal, protistocidal and fungicidal action of phytocides by Dr. J. C. Ray, M.D., F.N.I., Director, Indian Institute for Medical Research, Calcutta.*

The Committee discussed the work done by Dr. Ray and recommended that he should restrict his research to the study of pharmacology and toxicity of the active principles which he has already isolated with a view to explore their therapeutic value. The Committee also desired that Dr. Ray should send a selected list of plants which in his opinion were worth exploring.

(ii) *Enquiry on the action of snake venoms and their constituents on the biochemical activities of tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.*

The Committee recommended that the enquiry be continued.

The Committee decided to recommend the following enquiries in order of priority as under :—

1. Enquiry by Sir Ram Nath Chopra.
2. Enquiry by Dr. J. C. Gupta.
3. Enquiry under Dr. B. N. Ghosh.
4. Enquiry by Dr. J. C. Ray.

The meeting concluded with a vote of thanks to the Chair.

OTHER RESEARCHES.

1. Filterable viruses enquiry under the Director, King Institute, Guindy, Madras.

A. Influenza virus.

Influenza virus vaccine.—The potency of the influenza virus vaccine was studied by the mouse-protection tests soon after its manufacture and at the intervals at two months, four months and eleven months. The vaccine tested after two months and four months was potent and that tested after eleven months was not potent.

Diagnosis of influenza.—Isolation of the virus could not be attempted for want of suitable cases. There were no institutional outbreaks during the year and the cases referred from the city hospitals were too late for the isolation of the virus. However, serum tests were done on a few hospital cases diagnosed clinically as influenza for evidence of infection with the virus. As the sera did not inhibit fowl red cell agglutination by influenza virus in significant dilution it was presumed that the cases were not of virus influenza. Serum tests were also done with normal sera. The results are shown in Table I. It will be seen that normal human serum inhibits agglutination at a dilution of 1 : 128 to 1 : 256 ; some sera giving a titre of 1 : 1024. In the diagnosis of virus influenza significance is attached to the increase in the serum dilution causing inhibition of agglutination of chick cells during the course of illness which must be at least 4 fold.

Table I.

Agglutination-inhibition titre of human sera with influenza viruses A and B.

Antigen.	Below.						
	64	64	128	256	512	1,024	Total.
A ...	9	26	41	24	10	5	115
B ...	9	12	17	23	6	2	69

B. Effect of bacterial enzymes on virus receptors.

The phenomenon of adsorption of the virus influenza by erythrocytes of various species of animals and its subsequent elution under certain conditions is of general interest in the problem of the mechanism of infection by viruses since the red cell virus system may well involve principles which apply to the virus-host cell relationship. The elution of the virus from cells, accompanied by the disappearance of the virus-adsorbing capacity of the cells, indicates that an active destructive process has occurred. It has been suggested by Hirst (1942) that the destruction of the receptors is due to an enzyme possessed by the virus which is active against a substrate possessed by the cell.

In view of the enzyme-like character of the action of influenza viruses on red cells, attempts were made to study the effect of certain enzyme preparations from different bacteria and cholera vibrios for similar activity.

The organisms taken up for study were :—

(1) *Staphylococcus aureus.*

(2) *Staphylococcus albus.*

(3) *Streptococcus pyogenes.*

(4) *B. coli.*

(5) *Vibrio cholerœ.*

The cultures were grown on agar slopes. After 24 hours' incubation at 37°C. the cultures were emulsified with normal saline, frozen and kept in the refrigerator overnight. It was then allowed to thaw and lightly centrifuged. The supernatant was filtered through Seitz pads, tested for sterility and kept in the refrigerator.

In the cases of the *V. cholerœ*, the Seitz filtrate was heated at 55°C. for 40 minutes in the presence of excess calcium chloride to render it non-hæmolytic.

The allantoic cavity of 13-day-old embryos was drained and re-filled with 5·0 c.c. of the culture filtrate, and the eggs were slowly rotated and incubated at 37°C. for 30 minutes. At the end of the period the fluid inside the eggs was removed and a further 5·0 c.c. of saline containing 0·5 per cent formalin and influenza A virus was introduced. The eggs were then incubated at 37°C. Samples of the fluid from the allantoic cavity were taken at regular intervals over a period of 3 to 4 hours and titrated for the hæmagglutinin content.

Control eggs received only an initial treatment with normal saline in the place of culture filtrate. The necessary data are incorporated in Table II :—

TABLE II.

Enzyme preparation from	Titration of the samples of fluid from allantoic cavity at the end of :				
	30 minutes.	60 minutes.	120 minutes.	180 minutes.	240 minutes.
Staphylococcus albus	1 : 4	1 : 32	1 : 64	1 : 192	1 : 256
Staphylococcus aureus	1 : 8	1 : 32	1 : 96	1 : 128	1 : 192
Streptococcus pyogenes	1 : 4	1 : 16	1 : 64	1 : 128	1 : 256
Escherichia coli ...	1 : 4	1 : 32	1 : 64	1 : 128	1 : 128
V. cholerœ ...	1 : 128	1 : 192	1 : 128	1 : 192	1 : 256
Control ...	1 : 4	1 : 32	1 : 64	1 : 128	1 : 256

It will be seen that none of the bacterial enzymes taken up for study except that of *V. cholerœ* had any effect on the adsorption of influenza virus by susceptible cells.

In the control eggs there is a rapid adsorption of the virus followed by gradual liberation after a period of 3 to 4 hours. Pre-treatment with the enzyme from the *V. cholerœ*, however, prevents this initial adsorption.

C. POLIOMYELITIS VIRUS.

Attempts were made to isolate poliomyelitis virus from the stools by infecting monkeys intranasally with the stool suspension of cases clinically diagnosed as poliomyelitis.

Sinicus monkeys readily available locally, were used in our experiments. A 10-per cent suspension in Tyrode's solution of a representative sample of stool

collected on three successive days was made. The suspension was centrifuged at 1,500 r.p.m. for 15 minutes to remove the coarser particles. Under light ether anæsthesia the nasal mucous membrane of the monkey was gently traumatized by introducing a pipe cleaner into the nostrils. 5·0 c.c. of the stool suspension in Tyrode was then instilled into the nostrils, 2·5 c.c. being introduced slowly into each nostril with a syringe. Two monkeys were used for each specimen.

The animals were given three doses at intervals of 1 to 5 days. Morning and evening temperatures were recorded daily and the animals observed carefully for the development of muscular weakness or paralysis.

Specimens from 5 cases were taken up for study. Monkeys infected with stools from 3 cases did not develop any symptoms. Monkeys infected with the stool suspension from the other two cases developed paralysis. The sections of the spinal cord showed chromatolysis in cells. Monkeys infected with a 10 per cent suspension of the spinal cord of the infected monkeys also devoloped symptoms. Parts of the cord are sectioned for histological study and the rest preserved for passage of the virus.

A strain of the virus isolated is being maintained and its property studied.

Car Nicobar (*Lieut.-Colonel. C. C. Kapila's specimens.*)—Samples of stools collected from cases of poliomyelitis in an epidemic in Car Nicobar were sent by Lieut.-Colonel C. C. Kapila. The stools were pooled and two monkeys were infected (one *sinicus* and the other *rhesus*) intranasally in the usual manner. The monkeys did not show any symptoms.

Sinicus monkeys infected with the Tyrode suspension of the cord material of a fatal human case of poliomyelitis also sent by Colonel Kapila developed the disease. The virus is now in its fifth passage and its properties are being studied.

D. Vaccinia virus.

Two strains of vaccinia virus cultivated on the chorio-allantoic membrane of the developing chick are being maintained. One strain is in the 132nd passage and the other is in the 31st passage.

2. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

1. Isolation of the inhibitor of the cytochrome-oxidase system (warburg's respriatory enzyme) from cobra venom.

In the previous report, sufficient evidence was recorded regarding the existence of a separate active principle in cobra venom which is responsible for the inhibition of the cytochrome-oxidase system in tissue cells. Attempts were made to isolate this inhibitor from the crude venom and a method was suggested for the partial purification of the inhibitor. The method has been further improved and modified so as to yield a product, which for the same weight, is about 16 times more active than the crude venom. The method that has been finally adopted is as follows :—

The partially purified product (obtained by the method described in the previous report) was dissolved in 10 c.c. of water, the pH adjusted at 6·0, the solution cooled to 1°C. and treated with 15 c.c. of a saturated $(NH_4)_2SO_4$

solution, and then filtered off. The precipitate was again dissolved in 10 c.c. of water, the pH adjusted at 4·0, the solution cooled in ice and 8·5 c.c. of a saturated solution of $(NH_4)_2SO_4$ were again added. The precipitate was allowed to settle for 30 minutes in the frigidaire and then filtered off. This precipitate was dried. The precipitate contained 33 per cent of the total activity of the starting material and its protein content was 2·12 per cent with respect to crude venom. It was, therefore, 33/2·12 or 16 times more active than the crude cobra venom. The toxicity of the product, after removal of $(NH_4)_2SO_4$ was determined by intramuscular injection into pigeons and was found to be 0·3 m.

The properties of the inhibitor isolated from crude cobra venom.—With a view to throwing some light on the chemical nature of the inhibitor of cytochrome-oxidase system, the effects of varying temperature, pH and ultra-violet rays on its stability were investigated. To study the effects of heat, 0·06 per cent solutions of the inhibitor in phosphate-Ringer were exposed to different temperatures for 30 minutes. The solutions were then quickly cooled diluted suitably and its activity determined by measuring its effect on the oxidation of p-phenylene-diamine by pigeon's brain suspension. It was found that the inhibitor began to lose its activity at about 75°C. and was completely inactivated at 83°C; the half inactivation temperature was found to be 80°C. In order to ascertain if this inactivation by heat was reversible or not, the partly inactivated solutions were kept in the frigidaire at 4°C. for 48 hours and the activity determined again. No recovery of activity was observed even after 48 hours, thus showing that the inactivation was irreversible in nature.

The effect of different H-ion concentrations on the stability of the inhibitor was investigated by keeping 0·06 per cent solutions of the inhibitor at different pH values in a frigidaire at 4°C. for a period ranging from 1 to 3 days. The different pH values at which the inhibitor solutions were adjusted were 1·0, 3·0, 5·0, 6·0, 7·4, 9·6 and 13·0. The stability of the inhibitor was found to be maximum at pH 7·4, and was reduced above and below 7·4, becoming very low at pH 13·0, at which it was inactivated in the course of a single day. This inactivation was found to be irreversible in nature.

To study the effect of ultra-violet rays, a 0·06 per cent solution of the inhibitor in phosphate-Ringer was kept at a distance of 30 cm. from a mercury vapour lamp for a period of 15 minutes to 2 hours. Another solution of the same concentration was kept unexposed to serve as control. It was found that ultra-violet rays had no effect on the stability of the inhibitor.

The molecular weight of the inhibitor of cytochrome oxidase was determined by studying its rate of diffusion through a porous diaphragm. The molecular weight of the inhibitor, as calculated from the radius of the particles, was found to be 43,180.

The nature of the electrical charge and the migration of the particles of the inhibitor of the cytochrome-oxidase system at different pH values were investigated by studying the transport of the protein in an electric field set up in a U-tube. The iso-electric point of the inhibitor was found to be in the neighbourhood of pH 8·6.

With a view to elucidating the mechanism of action of the inhibitor, its effect on the cytochrome-cytochrome oxidase system was compared with that produced by cyanide and H_2S. It was found that whilst H_2S and cyanide were almost instantaneous in producing their effects, the inhibitor (isolated

from cobra venom) was very slow in its action. The concentrations of cyanide and H_2S necessary to produce complete inhibition were found to be of the order of 10^{-6} and 10^{-4}M respectively, whereas the concentration of the inhibitor necessary to produce the same effect was 5×10^{-8}M, i.e. the inhibitor was about 20 times more active than cyanide. It has been established by spectroscopic investigations that cyanide and H_2S inhibit the action of cytochrome-oxidase by combining with the iron of the respiratory enzyme. Similar investigations with the inhibitor isolated from cobra venom are necessary to elucidate its mechanism of action.

2. Effect of banded krait venom on the ærobic oxidation of some biologically important metabolites by tissue cells.

In the previous reports, experimental evidence was recorded regarding the effect of cobra venom on the ærobic and anærobic oxidation of different substrates like glucose, lactic acid, succinic acid, pyruvic acid, *p*-phenylene-di-amine, etc. It was reported that cobra venom, in minute doses, can inhibit strongly the oxidation of all the important substrates in presence of air; but when the oxidation was carried out in the anærobic phase by Quastel's ferricyanide technique (*Biochem. Jour.*, 1938, **32**, p. 936), it was found that the oxidation of succinic acid was very little affected by doses of cobra venom, which were sufficient to produce more than 80 per cent inhibition in the ærobic phase. From the experimental data, it was evident that Chain's suggestion of the breakdown of co-enzyme I by cobra venom (*Biochem. Jour.*, 1939, **33**, p. 407) could not explain the facts, and the existence of a separate active principle in cobra venom, which markedly affects the cytochrome-cytochrome oxidase system, was postulated.

It was considered necessary to investigate the effects of the venoms of the other poisonous snakes on the oxidation of different metabolites by tissue cells. The results obtained with banded krait venom on the oxidation of glucose were given in the previous report. The effect of the krait venom on the ærobic oxidation of the other substrates has also been investigated.

The technique of experiment was the same as was described in the case of the oxidation of glucose. Immediately after killing a pigeon, its brain (cerebrum and cerebellum) was taken out, cooled in ice and chopped to a fine paste. One hundred mg. of the paste were separately weighed out in several Warburg's flasks and suspended and broken up in phosphate-Ringer, pH 7·4. Adequate amount of the krait venom, also dissolved in phosphate-Ringer, was added to the brain suspension in some of the flasks, while the others served as controls. 0·5 c.c. of 1·2 per cent solutions of the substrates in phosphate-Ringer was kept in the side tubes in the case of sodium lactate and sodium succinate, whilst in the case of *p*-phenylene-di-amine, 0·5 c.c. of a 0·5 per cent solution of the hydrochloride in phosphate-Ringer and brought to pH 7·0, was used. Suitable controls were also kept. The total volume of liquid in each flask was 3·0 c.c. 0·2 c.c. of a 20 per cent solution of KOH was kept in the inner cup of each flask together with a roll of filter-paper. The flasks were fitted to the manometers and shaken for 1 hour and 30 minutes in presence of air in a thermostat bath kept at 37·5°C. During this period, the venom was given sufficient time to exert its maximum effect on the brain cells and moreover, a large portion of the substrates originally present in the brain cells was removed by oxidation. The substrate solution was then tipped off into the main compartment and the oxygen

consumption determined for 1 hour at intervals of 15 minutes. The results are recorded in the Table :—

TABLE.

Substrate used.	Mg. of venom per c.c. of brain suspension.	Percentage inhibition of oxidation.
Sodium lactate (0·2 per cent) ...	0·00033	45
	0·00067	67
	0·0013	80 to 90
Sodium succinate (0·2 per cent)	0·0033	65
	0·0067	75
	0·0130	90
P-phenylene-di-amine (0·0833 per cent)	0·0017	50
	0·0033	78
	0·0067	Over 90

From the results recorded in the Table, it will be evident that the venom of the banded krait, like that of the cobra, possesses the property of inhibiting the oxidation of the substrates by brain tissue. Moreover, since the krait venom is found to inhibit strongly the oxidation of *p*-phenylene-di-amine, it naturally contains an inhibitor of the cytochrome-oxidase system (Warburg's respiratory enzyme) and thus resembles cobra venom in this respect. It is not possible, however, at this stage to state whether banded krait venom has any effect on the individual respiratory enzymes like lactic de-hydrogenase, succinic dehydrogenase, etc. Further experiments on the effects of krait venom on the anærobic oxidation of lactic acid succinic acid, pyruvic acid, etc., by Quastel's ferricyanide technique are necessary to elucidate these points.

V. THE INDIAN JOURNAL OF MEDICAL RESEARCH and INDIAN MEDICAL RESEARCH MEMOIRS.

1. (a) *The Indian Journal of Medical Research, Volume XXXV*, 1947.—The usual four numbers of the *Journal* were published and these appeared in January, April, July and October respectively. The pre-war "standard" style of printing with wider margins and short title of papers and names of authors printed on the top of the page has been resumed since the beginning of this year. Tables also have now vertical rulings as before. The average size of each number was 85 pages of printed and tabular matter, with two plates, representing a decrease of 78 pages per number as compared with Volume XXXIV. The size of the present Volume with 4 numbers was, however, 12 pages more than that of the previous Volume with 2 numbers only.

(b) *The Memoirs.*—No *Memoir* was published with Volume XXXV.

(c) *Cost.*—The net cost of publication of Volume XXXV (four numbers) was Rs. 3,608.

2. (a) *The Indian Journal of Medical Research, Volume XXXVI*, 1948.—Only one number, i.e. the January 1948, has so far appeared. The April number is not expected to be out before early October. July and October numbers, both of which are already in Press may not be out before the end of this year. The reason for these inordinate delays is apparently the depletion of printing staff and other unsettled conditions in Calcutta. January issue contained 72 pages and 1 plate, April issue will have 112 pages and 2 plates, while July and October issues will have approximately 80 and 115 pages and 2 plates each, respectively. Volume XXXVI will, therefore, be 40 pages larger than Volume XXXV. There is now no dearth of suitable material and it is hoped we will be able to attain to our normal size of 160 pages per issue next year.

(b) *The Memoirs.*—As no suitable material was received, no *Memoir* was published with this Volume, but it is probable we may publish one early next year.

3. *Distribution* (1948).—

Indian subscribers	The figures for these have been called for and will be given in next year's statement. The total will be approximately 200.	
Foreign subscribers		
Exchange List	190	=317
Free List	127	
Voucher copies to advertisers, regn., make-up, police, specimen, propaganda, etc.		35
Balance left with publishers for sale ...		148
Total printed ...		700

4. *Distribution lists.*—The existing lists were last revised in July 1938. The revision of these lists is in hand but will not be completed before the end of the year by which time the position with regard to suspended institutes will be known.

5. *Number of copies printed.*—Only 700 copies of the *Journal* have been printed since 1940 instead of the previous 900. This number is considered sufficient to meet all current demands. Vols. I-VIII of the *Journal* are out of print.

6. *Cost of printing the Journal.*—The following statement shows the cost of production during the last five years:—

Cost of printing.

Number	XXXI 1943	XXXII 1944	XXXIII 1945	XXXIV-XXXV 1946-47	Number	XXXV-XXXVI 1947-48
	Rs.	Rs.	Rs.	Rs.		Rs.
May ...	2,633	2,116	4,037	4,086	April 1947	2,274
					July 1947	3,731
October ...	2,486	2,752	2,777	2,778	Oct., 1947	3,065
			Jan. '47	1,447	Jan. 1948	1,907
Total	5,199	4,868	6,814	8,311		10,977
Average pages	*125	*126	*160	†122		‡93

*Two numbers only †Three numbers (1946-47). ‡Four numbers (1947-48).

Recoveries.

	Rs.	Rs.	Rs.	Rs.		Rs.
May ...	837	1,293	1,842	1,204	April	705
					July	3,102
October ...	880	Nil	1,580	1,204	October	3,102
			Jan.	Nil	January	Nil
Total ...	1,717	1,293	3,422	2,408		6,909

7. *Net cost of Journal.*—The following statement shows the net cost of the production of the *Journal* during the last five years:—

Journal.

Number	1943	1944	1945	1946-47	Number	1947-48
	Rs.	Rs.	Rs.	Rs.		Rs.
May ...	1,796	823	2,194	2,882	April	1,569
					July	629
October ...	1,606	2,752	1,197	1,574	October	—37
			Jan. '47	1,447	January	1,907
Total ...	3,402	3,575	3,391	5,903		4,068

The net cost during 1947-48 after deduction of receipts was Rs. 4,068. The Publishers' bills for 1947-48 were submitted in time to make payments within the financial year.

8. *Financial position for the year* 1948-49.—The budget for the year provided for Rs. 14,000 to meet the cost of four quarterly numbers of the *Journal* and Rs. 2,000 for any *Memoir* which may be published. Estimated expenditure during the year is as follows:—

Cost of printing :—

	Rs.
Volume XXXVI, Nos. 2, 3 & 4 (April, July & October 1948) @ Rs. 3,250 each	13,000
Volume XXXVII, No. 1 (January 1949) @ Rs. 3,250) ...	
Total ...	13,000

Saving anticipated Rs. 3,000 but this may be reduced by Rs. 1,500 in case the *Memoir* under consideration is published during the current financial year.

9. *General.*—The editorial policy of the *Journal* was revised in 1945 on the recommendations of the Scientific Advisory Board and the Editorial Board was enlarged to include 25 referees. A note for the guidance of members of the Board was also prepared by the Editor and circulated to all concerned. The services of the members of the Board have been fully utilized in accordance with the policy adopted. The list of members on the Editorial Board has thinned considerably due to many officers having retired. It is being brought to its original strength by inclusion of new members and proposals for this are under consideration at present.

As decided by the Scientific Advisory Board publication of quarterly issues of the *Journal* has already been resumed with effect from January 1947. Now that the paper shortage is not so acute, we have reverted to the old style of printing with wider margins and narrower columns.

The unusual delay in the appearance of the *Journal* is due to the difficulties experienced by the Publishers as a result of strikes and unsettled conditions generally in Calcutta and their helplessness in working to any schedule. It is not due to dearth of suitable material, nor because the MSS do not reach the Publishers in time. I am inclined to think that their troubles are to some extent genuine and often unavoidable and in view of their past satisfactory services extending over a period of 34 years, it would be unwise to change the Printers at this stage. The July and October numbers will appear simultaneously. Every effort is being made to bring out the numbers in time in future.

All the papers published in Vol. XXXVI were submitted by workers in India or were the results of investigations carried out in India. The lines of special research activity are reflected in the nature of papers received. Of the 39 articles which have been published or accepted for publication, 13 were on nutrition, 10 on bacteriology, 5 on biochemistry, 3 on rabies, 2 on snake venoms, 2 on pharmacology, 3 on hæmatology and 1 on water-supplies.

The size of the *Journal* was considerably reduced owing to reduction in the number of papers received for publication during the last 5 years, but it is hoped that with the revival of research in India enough material will be forthcoming to bring the *Journal* to its pre-war size.

VI. INDIAN JOURNAL OF MALARIOLOGY.

The Indian Journal of Malariology, Volume I, Nos. 3 *and* 4, 1947.—Owing to the disturbed conditions prevailing in the country during the latter part of 1947, the publication of numbers 3 and 4 of this Volume was delayed. Both these issues consisted of a total of 196 pages of printed and tabular matter inclusive of plates. The September number consisted of seven articles dealing entirely with therapeutic trials on paludrine carried out in different parts of the country.

The cost of publication of Volume I slightly exceeded the sanctioned amount (Rs. 10,000) and this was due to a greater volume of published matter in the March issue.

The Indian Journal of Malariology, Volume II, 1948.—Owing to the fewer number of articles received late for publication and due to the disturbed conditions as referred to above, the March and June numbers of this Volume were combined. The September issue is in press and is likely to be ready for distribution in November.

Distribution.—There is a slight reduction in the number of copies distributed in the exchange and free list, but on the other hand the number of Indian and Foreign subscribers has shown an appreciable increase.

Indian subscribers	130		
Foreign subscribers	60	=	190
Exchange list	128		
Free list	82	=	210
Voucher copies to advertisers, specimens, propaganda, etc.			12
At the Malaria Institute of India for distribution ...			30
Balance left with publishers for sale			128
Total printed			600

VII. PAPERS PUBLISHED ON MEDICAL RESEARCH UNDER THE AUSPICES OF THE INDIAN RESEARCH FUND ASSOCIATION DURING THE YEAR 1948.

A. *Indian Journal of Medical Research* and *Indian Medical Research Memoirs.*

1. AHUJA, M. L., and GURKIRPAL SINGH.—Observations on Cholera Vaccine (Vol. 36, No. 1).
2. BANERJEE, S., GHOSH, N. C., and BHATTACHARYA, G.—Studies on the Effect of Nicotinic Acid on the Blood Sugar and Urinary Excretion of Sugar on Normal and Diabetic Rabbits (Vol. 36, No. 4).
3. DE, H. N., and BANERJEE, K. C.—Nicotinic-acid Requirements of Indian Adult (Vol. 36, No. 4).
4. DE, H. N., and CHAKRAVORTY, C. H.—Ascorbic-acid Requirement of Indian Adult (Vol. 36, No. 3).
5. DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Utilization of Soya-milk Protein for the Formation of Blood Proteins (Vol. 36, No. 2).
6. DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Protein Value of Soya-bean Milk : Human Feeding Experiments (Vol. 36, No. 2).
7. GEORGE, MARIAM, and PANDALAI, K. M.—On the Nature of Penicillin Bacteriostasis. Part I. Nucleic Acid Antagonism of Penicillin Bacteriostasis (Vol. 36, No. 3).
8. GEORGE, MARIAM, and PANDALAI, K. M.—On the Nature of Penicillin Bacteriostasis. Part II. Relation between Penicillin Action and the Gram-staining Characteristics of certain Pathogens (Vol. 36, No. 3).
9. GEORGE, MARIAM, and PANDALAI, K. M.—Synergism in Chemotherapy. Part II. Further Studies on Penicillin-dye Synergy upon Gram-negative Bacteria.
10. GOYAL, R. K.—Estimation of the Immunizing Potency of Antirabic Vaccines (Vol. 36, No. 2).
11. GOYAL, R. K.—Mode of Circulation and Excretion of Toxins, Antitoxins, Venoms and Bacteria in the Animal Body (Vol. 36, No. 3).
12. KARNANI, B. T., DE, S. S., and SUBRAHMANYAN, V.—Fortification of Soya-bean Milk with Calcium and Study of its Availability to Young Growing Rats (Vol. 36, No. 4).
13. KARNANI, B. T., DE, S. S., and SUBRAHMANYAN, V.—Relative Utilization of Calcium from Soya-milk (fortified with dicalcium phosphate) and Cow's Milk by Growing Children (Vol. 36, No. 4).
14. MISRA, U. C., and PATWARDHAN, V. N.—The Nutritive Value of Hydrogenated Vegetable Oils. The Digestibility of Ground-nut (*Arachis hypogea*) Oil Hydrogenated to different Degrees of Hardness (Vol. 36, No. 1).
15. MUKUNDAN, R., and RAMA SASTRI, B. V.—A Simple Method for the Removal of Interfering Substances in the Estimation of Thiamine in Urine (Vol. 36, No. 4).
16. RANGNEKAR, Y. B., DE, S. S., and SUBRAHMANYAN, V.—Soya-bean Ascorbicase (Vol. 36, No. 4).

17. SHRIVASTAVA, D. L., GURKIRPAL SINGH, and AHUJA, M. L.—Immuno-chemical Studies of *Vibrio cholerœ* (Vol. 36, No. 4).

B. *Indian Journal of Malariology.*

1. CHAUDHURI, R. N., and CHAKARAVARTY, N. K.—Clinicals with Cam-Aqi in Malaria (Vol. 2, No. 3).
2. CHAUDHURY, R. N., RAI CHAUDHURI, M. N., and CHAKRAVARTY, B. M.—Chloroquine (S.N. 7618 in Malaria) (Vol. 2, Nos. 1-2).
3. DAKSHINAMURTY, S.—Measurements of Malaria, Part II (Vol. 2, No. 3).
4. JASWANT SINGH.—Engineering and Man-made Malaria (Vol. 2, No. 3).
5. PURI, I. M.—The Distribution of Anopheline Mosquitoes in India, Pakistan, Ceylon and Burma, Part V. (Vol. 2, Nos. 1-2).
6. RAMAKRISHNAN, S. P., and RAMAKRISHNA, V.—A Note on the Choice of Spraying Equipment in Malaria Control with DDT Residual Spraying (Vol. 2, No. 3).
7. RAY, A. P.—Prophylactic Use of Paludrine in a Tea-estate (Vol. 2, Nos. 1-2).

C. *List of papers published by workers under the I.R.F.A. in Journals other than the "Indian Journal of Medical Research" and the "Indian Journal of Malariology".*

1. CHAUDHURI, R. N., and CHAKRAVARTY, H.—Intravenous Paludrine (*Brit. Med. Jour.*).
2. CHAUDHURI, R. N.—Treatment of Malaria (*Ind. Med. Gaz.*, May 1948).
3. DAMODARAN, M., and YESHODA, K.—Amino Acids and Proteins in Haemoglobin Formation (*Biochem. Jour.*, 1947).
4. DHARMENDRA.—Planning of Leprosy Work in India (All-India Leprosy Workers' Conference, Wardha).
5. DHARMENDRA.—Need for Fresh Anti-leprosy Legislation in India (All-India Leprosy Workers' Conference, Wardha).
6. DHARMENDRA.—The Place of Vasectomy in Leprosy Control (All-India Leprosy Workers' Conference, Wardha).
7. DHARMENDRA.—Classification of Leprosy (All-India Leprosy Workers' Conference, Wardha).
8. DHARMENDRA and CHATTERJEE, K. R.—Treatment of Leprosy with Sulphone Drugs (All-India Leprosy Workers' Conference, Wardha).
9. DHARMENDRA, MUKERJEE, N., and CHATTERJEE, S. N.—A Follow-up Study of Reacting Tuberculoid Lesions (International Leprosy Congress, Cuba).
10. DHARMENDRA, CHATTERJEE, S. N., and MUKERJEE, N.—A Follow-up Study of Leprosy Cases of 'Doubtful' Classification (International Leprosy Congress, Cuba).
11. DHARMENDRA, and MUKERJEE, N.—Cockroaches not Responsible for Transmission of Leprosy (International Leprosy Congress, Cuba).
12. DHARMENDRA.—Treatment of Leprosy with Sulphone Drugs (International Leprosy Congress, Cuba).

13. DHARMENDRA.—Classification of Leprosy (International Leprosy Congress, Cuba).

14. DHARMENDRA and SEN, N. R.—Frequency of the Presence of Leprosy Bacilli in Nasal Smears of Leprosy Patients (International Leprosy Congress, Cuba).

15. DHARMENDRA and MUKERJEE, N.—Effect of Sunlight on the Staining Properties of Leprosy Bacilli (International Leprosy Congress, Cuba).

16. PATWARDHAN, V. N.—Mode of Action of Vitamin D—A Résumé (*Curr. Sci.*, Vol. 17, 1948).

17. PATWARDHAN, V. N.—The Nutritive Value of Hydrogenated Oils (Vanaspati) (*Jour. Sci. & Ind. Res.*, Vol. 7, 1948).

18. PATWARDHAN, V. N., and RAMALINGASWAMI, V.—Nutritional Deficiency Diseases in India with Demonstration of Cases (*Jour. Christ. Med. Assoc.*, July 1948).

19. RAMALINGASWAMI, V., VENKATACHALAM, P. S., and MENON, P. S.—Infantile Pellagra : a Report on Five Cases (*Ind. Phys.*, Sept. 1948).

20. RAMALINGASWAMI, V.—Nutritional Diarrhœa in Vitamin A Deficiency (*Ind. Jour. Med. Sci.* Nov. 1948).

21. Observations on the Carotenoids Pigments of Different Varieties of Oranges (*Jour. Sci. & Ind. Res.* 1948).

22. CHITRE, R. G., and DESAI, D. B.—Nutritive Value of some Pure Strains of Cereals and Pulses (*Ind. Jour. Med. Sci.*, Vol. 2, 1948).

23. CHITRE, R. G., DESAI, D. B. and BHARANI, S. P.—Some Rich Sources of Active Carotenoid (*Jour. Sci. & Ind. Res.*, Vol. 6, 1947).

VIII. PROGRAMME OF RESEARCHES AND MISCELLANEOUS GRANTS RECOMMENDED FOR THE YEAR 1949-50.

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 3rd and 4th December, 1948.

Enquiries.

Cholera.

	Budget allotments. Rs.
Continuation enquiries :—	
1. Enquiry on cholera under the Director, Central Research Institute, Kasauli	17,533
2. Re-Orientated programme of cholera research.	80,000

Malaria.

Continuation enquiries :—

3. Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi	41,859
4. Enquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	10,341
5. Enquiry on the chemotherapy of malaria under Dr. K. Ganapathi at the Haffkine Institute, Bombay	27,301

New enquiries :—

6. Enquiry on sex determination in chrysomyia ruficacies under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta ...	5,615
7. Enquiry on complement-fixation test in Malaria for the assessment of anti-malarial drugs under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta	3,250

Nutrition.

Continuation enquiries :—

8. Nutrition Research Laboratories, Coonoor	2,04,749
9. Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay	21,128
10. Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta	26,329
11. Nutrition research unit under Professor V. Subrahmanyan and Dr. S. S. De at the Indian Institute of Science, Bangalore ...	7,954
12. Enquiry entitled 'Preparation of anti-rachitic substances' under Professor B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory, S. P. College, Poona	3,900

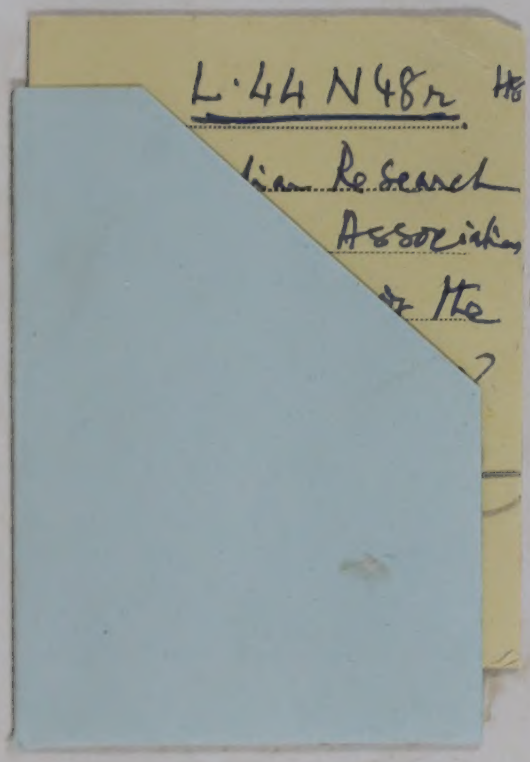
Enquiries.	Budget allotments Rs.
13. Enquiry on the metabolism of vitamin A under Dr. K. Rajagopal at the All-India Institute of Hygiene & Public Health, Calcutta	6,426
New enquiries :—	
14. Enquiry into the nutritive value of diets prescribed for hospital patients in India under Dr. K. Mitra, Adviser on Nutrition, Directorate-General of Health Services, New Delhi	4,470
15. Enquiry into the effects of malnutrition on the physique and more particularly on the blood picture including hæmoglobin and plasma-protein concentration as also the treatment thereof with high-protein diet hydrolysates, etc., under Professor (Miss) S. Chowdhury and Dr. K. Mitra at the Lady Hardinge Medical College, New Delhi	5,160
16. Studies on the biosynthesis of nicotinic acid under Dr. S. Banerjee, Head of the Department of Physiology, Presidency College, Calcutta	3,380
17. Enquiry on the physiological effect on dietary protein under Dr. M. Damodaran at the National Chemical Laboratory, Poona	13,000
Leprosy.	
Continuation enquiries :—	
18. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta	19,489
19. Study of histopathology of leprosy under Dr. R. G. Cochrane at the Government Lady Willingdon Leprosy Sanatorium, Chingleput	19,875
Plague.	
Continuation enquiries :—	
20. Plague researches under the Director, Haffkine Institute, Bombay	25,417
21. Plague enquiry at Coonoor in the Nilgiris district under the Director of Public Health, Madras	5,050
22. Plague enquiry under Dr. R. B. Lal at the All-India Institute of Hygiene & Public Health, Calcutta	16,392
Clinical research.	
Continuation enquiries :—	
23. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	10,060
24. Neuropathological unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	40,000
25. Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay	14,045
26. Enquiry on typhus under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta	17,110

Enquiries.	Budget allotments Rs.
27. Enquiry into the effects of vitamins B and C in diabetes under Dr. S. Banerji at the Presidency College, Calcutta	10,000
28. Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	13,500
29. Haematological unit under Dr J. C. Patel at the Seth G. S. Medical College, Bombay	12,795
30. Enquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval, Serologist to the Government of India, School of Tropical Medicine, Calcutta	5,730
31. Enquiry on the clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Mr. B. N. Banerji and Dr. V. R. Naidu at the Indian Institute of Science, Bangalore and Sri Krishnarajendra Hospital, Mysore	12,000
32. Enquiry to investigate allergic diseases and significance of endogenous allergens as regards aetiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta	9,194
33. Enquiry on the immunological properties of lens and uveal protein under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay	7,950
34. Enquiry on the neurogenic factor in the production of traumatic shock and its treatment by the alteration of cerebrospinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay	8,670
35. Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin under Dr. B. Mukerji at the Central Drugs Laboratory, Calcutta	20,000
New enquiries :—	
36. Grant to conduct clinical trials with the drug 'V-187' under Dr. N. K. Sahiar and Dr. N. M. Purandare at the K. E. M. Hospital, Bombay	11,245
37. Enquiry on the state of refraction of Indian children of different communities with special reference to myopia under Dr. G. S. Pendse at the Telegaon General Hospital, Poona	3,660
38. Study of monilla infection of the skin and adjacent mucous membranes under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	8,875
39. Enquiry on the problem of typing typhoid bacilli by means of Vi bacteriophage and treatment of typhoid fever with type specific Vi 'phage under Dr. R. G. Dhayagude and Dr. D. D. Banker at the Seth G. S. Medical College, Bombay	2,660

Enquiries.	Budget allotments Rs.
40. Enquiry on the associated vaccine for prophylactic immunization against typhoid, paratyphoid, cholera, dysentery and tetanus by a single inoculation under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.	9,275
41. Enquiry on the circulatory changes in severe infection under Dr. R. N. Chaudhury at the School of Tropical Medicine, Calcutta	7,295
Maternity and Child Welfare.	
Continuation enquiries :—	
42. Enquiry on anæmia in pregnancy under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	2,700
New enquiries :—	
43. Enquiry into morbidity of children between the ages of 1-5 years (pre-school) in the Singur Health Centre areas under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta.	7,600
44. Enquiry into the vital losses of pregnancy and infancy in a rural population under Dr. Muktha Sen and Mr. K. K. Mathen at the All-India Institute of Hygiene & Public Health, Calcutta ...	2,200
Filariasis.	
Continuation enquiries :—	
45. Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta	8,001
46. Enquiry to test the usefulness of the intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras	7,681
47. Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. K. Venkataraman, Director of the Technology Department of the University of Bombay	12,890
New enquiries :—	
48. Cost of setting up a unit to conduct field trials for the control of filariasis under the Director, Malaria, Institute of India, Delhi ...	40,800
Industrial Health.	
Continuation enquiries :—	
49. Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta	45,665
50. Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta ...	6,600
51. Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar ...	2,090

Enquiries.	Budget allotments Rs.
52. Enquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose at the Universty College of Science & Technology, Calcutta	2,340
New enquiries :—	
53. Enquiry into the prevalence of byssinosis in cotton textile industry in South India under Dr T. S. Adisubramaniam, Madras Public Health Service, Madras	7,480
Pharmacology.	
Continuation enquiries :—	
54. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratory, Jammu	9,329
55. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta	29,088
Other researches.	
Continuation enquiries :—	
56. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	14,095
57. Enquiry on the action of snake venoms and their constituents on the biochemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta ...	4,810
58. Statistical enquiry into the numerical estimation of coliform organisms in water samples by the dilution method under Dr. Satya Swaroop, Statistical Officer, Public Health Department, East Punjab, Kasauli.	2,514
Grants.	
(i) Office of the Secretary, Indian Research Fund Association ...	1,06,000
(ii) Staff employed in the Office of the Accountant-General, Central Revenues, for I.R.F.A. work	10,000
(iii) I.R.F.A. reports and publications	13,000
(iv) Travelling allowance of the members of the Governing Body, Scientific Advisory Board and Advisory Committee of the I.R.F.A.	20,000
(v) Cost of two professorships at the School of Tropical Medicine, Calcutta	34,558
(vi) Contribution to the Imperial Institute of Entomology, London	1,350
(vii) Contribution towards the Bureau of Hygiene & Tropical Diseases, London	2,025
(viii) Research fellowships	96,000

	Grants.		Budget allotments Rs.
(ix)	*Indian Journal of Medical Research* and *Indian Medical Research Memoirs* :—		
	(a) Printing and publication charges of the *Journal*	14,000	
	(b) Printing and publication charges of *Memoirs*	2,000	
	(c) Editor's Office	10,050	
			26,050
(x)	*Indian Journal of Malariology* :—		
	(a) Printing and publication charges of the *Journal*	10,000	
	(b) Editor's Office	3,360	
			13,360
(xi)	I.R.F.A. library at the Central Research Institute, Kasauli ...		700
(xii)	I.R.F.A. microfilm service unit		11,493
(xiii)	Pay (including special pay and allowances) and contributions towards leave, salary and pension or contributory provident fund of officers of the Medical Research Department under the Association		75,000
(xiv)	Travelling allowances and certain contingent expenses for Medical Research Department officers who are employed under the I.R.F.A. and are attached to central or provincial laboratories		10,000
(xv)	Clerical assistance at the School of Tropical Medicine, Calcutta		8,595
(xvi)	Clerical assistance at the All-India Institute of Hygiene & Public Health, Calcutta		3,468
(xvii)	Travelling allowance for I.R.F.A. workers on transfer ...		3,000
(xviii)	I.R.F.A. contribution and interest towards the Contributory Provident Fund		32,000
(xix)	Miscellaneous unforeseen expenditure		60,000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